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THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSICOCHEMICAL PROPERTIES OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON [1]

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INTRODUCTION

The problem of the relationship between the composition and concentration of the soil solution and the characteristics of the plant is of such basic importance in crop production that it has attracted the attention of the ablest investigators since the beginnings of agricultural chemistry, notwithstanding whose efforts exact quantitative knowledge is evidently still in the earliest developmental stages.

One reason for this state of affairs is that the problem is so complex that it can not be solved by any one method. Admitting and emphasizing, as the writer does, the necessity for the use of various techniques in the disentanglement of the complicated network of interrelationships and in the study of the individual threads of which it is made up, it is his purpose in this paper to stress one hitherto neglected method of research by showing, on the basis of actual experimental results with one agricultural crop, that it is capable of yielding definite conclusions as to certain phases of the problem.

The method emphasized is that of the application of the modern higher statistics to the analysis of the results of measurements on the soil properties and on the plant characteristics of the different parts of the experimental field upon which the cultures are grown. If this method can be shown to yield valuable results, it has the great advantage of being applicable to experiments conducted primarily for other purposes and of dealing with plants under the field conditions which are wholly normal for their growth in practical agriculture.

The high degree of consistency and the high order of precision of the end results of the present experiments, which involved relatively crude measurements upon the characteristics of the soil, show clearly that with proper refinement of measurements and with proper increase in the scope of the experiments, these methods open up large possibilities for advance in our knowledge of the complex problem of the relationship between the properties of the soil and the characteristics of the plant. The illustrative materials are Egyptian and Upland cotton as grown under irrigation agriculture in the arid Southwest. The findings for the agricultural varieties of these two types of cotton constitute a contribution to the system of quantitative physiological

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data for this crop which is being built up to supplement various genetic and plant-improvement investigations (*18, 19, 20, 21*)[2] by the Office of Drought and Alkali Resistant Plant Investigations of the Bureau of Plant Industry of the United States Department of Agriculture, with the cooperation in certain of the present phases of the work of the Office of Biophysical Investigations and the Office of Western Irrigation Agriculture of the Bureau of Plant Industry. In the present paper, however, the data for the soil and for the plant serve equally to extend certain lines of investigation on the problem of the differentiation of plots in relation to the interpretation of crop yields which have been under consideration by the Office of Dry Land Agriculture and the Office of Western Irrigation Agriculture of the Bureau of Plant Industry (*9, 10*) for the past several years. While the papers just cited, and others which precede them (*4, 5, 6, 7, 8*), deal primarily with defects of agronomic experimentation as generally conducted, they also emphasize the necessity for, and the fruitfulness of, a statistical attack upon the problem of the analysis of plot yields and the relationship between the properties of the soil and the characteristics of the plant.

PURPOSE OF THIS INVESTIGATION

The specific purpose of the present paper is twofold: (*a*) to furnish further illustrations of the existence of substratum heterogeneity with respect to the physical factors of the soil in very small experimental fields, and (*b*) to demonstrate the possibility of the determination in the quantitative terms of the correlation coefficient of the relationship between the physical and chemical properties of the soil of different portions of the field and the character of the crop produced on it.

The first of these extends our hitherto inadequate knowledge of the definite physical factors to which the heterogeneity of the soil is due. The second furnishes further evidence concerning the amount of the influence of these factors upon the plant.

The possibility of measuring the relationship between soil properties and plant characters in the field is suggested by the demonstration in the papers cited above that experimental fields are, practically without exception, heterogeneous in the sense that they show differences throughout the field in capacity for crop production of such magnitude as to influence, in like manner, but not necessarily in like degree, the yields of adjacent small plots (*7, 9*), and that these differences are of a relatively permanent nature (*10*). It has long been evident that the realization of this possibility as a means of routine investigation would depend upon the possibility of obtaining for the soil measurements of variables of physiological importance. That this would ultimately be practicable was rendered probable by the demonstration (*9*) that soil-heterogeneity coefficeints for water content and for chemical composition of about the same order as those for the characters of crop plants (generally yield) are found.

The present investigation shows that the relationship between the concentration of the soil solution and the physicochemical properties of the leaf-tissue fluids may be measured readily under conditions of field experimentation. While the present study has been limited to

[2] Reference is made by number (italic) to "Literature cited," p. 646.

the electrical conductivity of the soil solution and certain properties of the leaf-tissue fluids, there is no reason why these methods should not cover a wider range of soil properties and be extended to the important economic characteristics of the plant, such as yield and quality of product. Such investigations are already under way.

MATERIALS AND METHODS

The present statistical investigation is based on the data of two experimental cultures grown at the Cooperative Testing Station in the Gila River Valley at Sacaton, Ariz., 1921–22.[3] It is merely noted here that the first experiment, in 1921, described in detail elsewhere (*11*), covered an irrigation plot 26.5 by 400 feet on which 7 rows of cotton, each containing 20 replications of groups of plants to be compared, were grown.

In 1922, the north halves of three plots of the standard size were combined to form a plot[4] 79.5 by 200 feet. This was planted to 24 rows, each divided into 18 sections of 10 feet each, the extra 20 feet being used for buffer plantings at the ends. These 18 sections formed 6 groups of 3 sections each. One of these was planted to 10 hills of Acala Upland, one to 10 hills of Pima Egyptian, and one to 10 hills of Meade Upland. These groups of three varieties were therefore replicated six times in the length (180 feet) of the cultural area.

Methods of sampling and tissue analysis for the experiment of 1921 have been indicated elsewhere (*11*). In 1922 two adjoining rows were combined, so that samples comprised tissues from the plants of 10-foot sections of rows 1 and 2, 3 and 4, 5 and 6, etc. Thus, for purposes of sampling, the field is divided into 12 × 18 subplots, each 10 feet long with 2 rows of 10 hills[5] each of plants.

All soil samples were taken by borings to a depth of 4 feet.[6] Bridge readings for first, second, third, and fourth feet of the soil were made separately.

In 1921 two soil borings separated by about 6 inches were taken within a foot and a half of the hybrid plants and the cores combined to give a series of 80 sets of samples, upon which 320 readings for individual layers were made.

In 1922 the borings were made between the 10-foot sections of adjoining rows constituting a subplot. One was made at the center of the subplot and the two others at about 3.3 feet to either side. Thus the soil of the field was sampled by 12 rows of borings, each comprising 54 cores, separated by about 3.3 feet. The three cores for each subplot were thoroughly mixed by grinding, after drying at about 100° to 110°. More representative sampling of the field

[3] The field and analytical determinations were carried out by the writer with the cooperation of W. F. Hoffman, Arnold H. Johnson, R. D. Evans, John V. Lawrence, Zonja Wallen Lawrence, and A. T. Valentine. The writer is indebted to Miss Edna K. Lockwood for supervising the great amount of work necessary to reduce the raw data to statistical constants.

[4] While the three half plots were separated by slightly raised dikes to permit basin irrigation, they were all treated exactly alike and may be considered as a unit.

[5] The culture was thinned to one plant per hill. Because of very unfavorable conditions in the early spring, many hills failed to produce seedlings, and early advent of root rot further reduced the number of available plants. The reduction of the stand was largely compensated, as far as the total crop production is concerned, by the large size of the plants which remained, but the number of plants from which leaf tissue was collected for analyses was generally much less than 20.

[6] Soil texture and water content rendered sampling at greater depths so difficult as to be impossible for series of the size necessary for a statistical treatment of the problems under investigation.

would seem difficult to obtain, but it is unfortunate that the series could not have been repeated at intervals throughout the season.

Since each of the six sections of 30 feet contains Acala, Pima, and Meade plants, there is a system of 72 measurements on the substratum and on the plants for the determination of the relationship between the concentration of the soil solution (as defined for the purposes of this investigation) and the properties of the plant-tissue fluids for each variety of cotton, these measurements being distributed uniformly over a plot 79.5 by 180 feet. For investigation of the soil alone there is a series of 216 sets of borings, each providing four measures of soil resistance for individual layers.

The only measure of the concentration of the soil solution which was at all feasible for such large series of determinations as those which are necessary for present purposes at the time the field work was done is that furnished by the resistance of the soil mass as determined by the soil bridge, described in its present form by Davis and Bryan (*2*). In making the determinations only the cup of the instrument was employed, with a superior source of the alternating current and bridge.

It would of course be theoretically possible to calculate the percentage of salts from the resistances as read from the bridge. This has not been done for the following reasons: (*a*) While the resistance of the soil mass furnishes only a very incomplete expression of the nature of the soil solution, it is probably reasonably comparable from one part of the field to another and so may serve as well for purposes of statistical treatment as any other value which might be derived from it by means of a formula or constant. (*b*) To transfer the soil resistances into any other measure of the concentration of the soil solution would require analytical studies of the salt constituents of the soil. It is hoped that such studies can be made later in series sufficiently large to permit statistical treatment comparable with that here employed for soil resistance. It seems best to withold discussion on the basis of constants in terms other than those of direct measurement until such directly determined analytical values are at hand.

The constants employed here are the resistances as read in the standard soil cup, corrected to standard temperature in accordance with the practice and tables of the Bureau of Soils of the United States Department of Agriculture. It is perhaps unfortunate that these were not expressed as reciprocal ohms, since the form would then have been comparable with that for the specific electrical conductivities of the plant-tissue fluids. The similarity would, however, have been one of form only, since the plant tissue fluids are expressed in terms of the specific electrical conductivity of standard solutions. This is not yet possible for conductivities as determined from the soil mass.

The foregoing pertains primarily to the distribution of the plantings and to the properties of the soil. A description of the plant characters adequate for the purpose of the present paper has been given elsewhere in connection with a discussion of the physicochemical properties (*11*), chloride content (*12*), and sulphate content (*15*) of the leaf-tissue fluids of Egyptian and Upland cotton.

RESULTS

The problems which require consideration fall into two groups: (*a*) That of the homogeneity or heterogeneity of the field upon which the cultures were grown. This merits further investigation on its own account. From the standpoint of the characteristics of the plant it is important to note that if the field selected for the experimental plantings does not differ in salinity from place to place, it is idle to attempt to investigate the relationship between soil resistance and the characters of the crops grown upon it. (*b*) That of the influence of soil salinity upon the various characteristics of the plant.

PHYSICAL HETEROGENEITY OF THE CULTURAL PLOTS

To determine to what extent the field is heterogeneous with respect to salt content, as measured by electrical resistance, the correlation between the resistances of samples from adjacent subplots has been computed.

The course followed in the analysis of the data for the soils of the two experiments has been somewhat different. In 1921 six different types of seed were used and only 80 sets of soil samples were taken. In 1922 only three types of seed were planted and sets of 216 soil samples, each of four individual foot layers, were available. The number of soil resistances and of determinations on plant-tissu fluids which may be considered in relation to each other for any one type of plants is very much larger in the experiment of 1922 than in that of 1921. The statistical analysis may therefore be safely carried to a higher degree of refinement. In dealing with the data for 1921 the statistical constants have been determined by the summation of the first and second powers and of the products of the actual physical constants (*3*) without grouping. In determining both the physical constants for the frequency distributions and the coefficients of correlation for the larger series of determinations in 1922 the soil resistances have been grouped in classes of 25 ohms range, as shown in the accompanying illustrative Tables I and II for the resistance of the first and second foot of soil of the subplots upon which Meade and Pima cotton were grown. Unfortunately the limitation of space precludes the publication of all the fundamental tables of data.

TABLE I.—*Correlation surface showing the relationship between the electrical resistance of the first foot (R_1) of soil of adjacent subplots on which Pima Egyptian and Meade Upland cotton were grown at Sacaton, Ariz., in 1922*

MEADE (R_1)	100	125	150	175	200	225	250	275	300	325	350	
325								1				1
300					1			1	1	1		4
275						1	1	6				8
250				1		5	1	1		1		9
225				1	6	7	2	1				17
200			1	3	2	2	1				1	10
175	1	3	2		2	3						11
150			2	1		1	1					5
125	1	1		1		1						4
100		1		1								2
75		1										1
Total	2	6	5	8	11	20	6	10	1	2	1	72

PIMA (R_1)

TABLE II.—*Correlation surface showing the relationship between the electrical resistance of the second foot (R_2) of soil of adjacent subplots on which Pima Egyptian and Meade Upland cotton were grown, at Sacaton, Ariz., in 1922*

MEADE (R_2)	PIMA (R_2) 50	75	100	125	150	175	200	225	250	275	300	325	350	375	400	425	450	475	
400												1		1					2
375														1			1		2
350										1	1			1					3
325									1	1	1								3
300								1		2			1					1	5
275		1	1		1									1					4
250				2					2			1							5
225			2			1	1		1										5
200				1		2	1	1		1									6
175						1		3		1	1								6
150			1	1			1			1									4
125	1		1	3	2		1	1	2										11
100	2		1	2			1			1									7
75		3	2	1	1	1													8
50	1																		1
Total	4	4	8	10	4	5	5	6	6	8	3	2	1	4			1	1	72

In determining the heterogeneity coefficient for the soil in the series of 1921, the 80 borings have been grouped by fours according to the 20 positions in the rows and the correlation between the readings in the different rows determined in two ways: First, the correlations between the borings of the individual rows have been determined independently in order to avoid the possible influence of the symmetry of the correlation surface (*6*)[7] upon the magnitude of the correlation coefficients as calculated by the usual formula (*7*). In this method of procedure the correlation between the resistances of associated soil samples in the first and third row, in the first and fifth row, in the first and seventh row and in the third and fifth row is determined, and so throughout the possible series of $\frac{1}{2}\ n\ (n-1)$ combinations. In no case has the correlation surface been made symmetrical. Second, the correlations determined by the usual heterogeneity coefficient (*7*) has been compared with the average of the coefficients for the individual rows.

ANALYSIS OF DATA FOR THE EXPERIMENT OF 1921

The constants measuring the relationship between the resistances of soil samples from comparable depths, and from the same section of the field but from different rows, appear in Table III. These show the irregularities which may be expected when only 20 pairs of measurements serve as a basis for the determination of a correlation but all are positive in sign, and the average values, however these are taken, are substantial magnitudes.

The averages of the six possible correlation coefficients given in Table III, measuring the relationship between the individual rows for the upper 4 feet of soil, and for the average resistance of the first to the fourth foot of soil, are laid beside the five constants determined by means of the heterogeneity coefficient in Table IV.

[7] This is a mathematical problem which will be discussed elsewhere. Here it is merely necessary to note that it is a factor amply covered by the method followed.

TABLE III.—*Correlation coefficients measuring the relationship between the salinity, as expressed in terms of electrical resistance, of the soil from various levels in neighboring borings in the experimental field at Sacaton, Ariz., in 1921*

	Second variable			Average
	Row 3	Row 5	Row 7	
First variable, soil of row 1:				
Resistance of first foot, R_1	0.815	0.680	0.339	0.611
Resistance of second foot, R_2	.558	.645	.441	.548
Resistance of third foot, R_3	.724	.703	.370	.599
Resistance of fourth foot, R_4	.312	.234	.398	.315
Average of all layers	.602	.566	.387	
Average resistance, R_{1-4}	.890	.768	.514	.724
First variable, soil of row 3:				
Resistance of first foot, R_1		.800	.499	.650
Resistance of second foot, R_2		.865	.168	.517
Resistance of third foot, R_3		.670	.368	.519
Resistance of fourth foot, R_4		.873	.596	.735
Average of all layers		.802	.408	
Average resistance, R_{1-4}		.881	.480	.681
First variable, soil of row 5:				
Resistance of first foot, R_1			.539	.539
Resistance of second foot, R_2			.266	.266
Resistance of third foot, R_3			.548	.548
Resistance of fourth foot, R_4			.472	.472
Average of all layers			.456	
Average resistance, R_{1-4}			.561	.561

These constants lead to the following conclusions:

(1) The coefficients are of a very substantial order of magnitude and statistically significant in comparison with their probable errors throughout. They fully confirm the conclusions drawn from other data (*9*) in showing that cultivated fields are characterized by a marked heterogenity of the physical and chemical characteristics of the soil as well as of the crops which they produce. This result is noteworthy because of the small size of the experimental plot (26.5 by 400 feet) on which the series of 80 borings was made.

(2) The averages for the various permutations of rows show that the correlations for continuous rows are higher than those for rows which are more widely separated. This is evident from an inspection of the constants for the individual soil layers, although these show considerable irregularities. A better conception of the relationship is furnished by an examination of the averages of the correlations for the individual soil layers, or of the coefficients for the average resistance of the four layers. Thus for the average resistance of the four layers it is found that the correlation for rows 1 and 3 is 0.890, as compared with 0.768 for rows 1 and 5, and 0.514 for rows 1 and 7. Similarly, the correlation for the borings of rows 3 and 5 is 0.881, as compared with 0.480 for rows 3 and 7. The average for rows 5 and 7 is 0.561, as compared with 0.514 for rows 1 and 7, and 0.480 for 3 and 7.

(3) The correlations for the different soil layers do not differ greatly among themselves, except as the differences represent irregularities in the correlation coefficients between the various combinations of rows.

(4) The average values of the coefficients measuring the relationship between individual pairs of rows [8] are in all cases slightly higher than those for all the possible combination of rows. Thus the entries in Table IV show that the constant for the first foot is 0.550 when deduced from a symmetrical table, whereas the average value is 0.612. In the second foot the value calculated by the usual heterogeneity coefficient is 0.349, whereas the average of the values obtained from pairs of borings from individual rows is 0.490. Similar results are found for soils from the other depths. This emphasizes again the necessity for a consideration of the influence of the symmetry of the correlation surface on the magnitude of the correlation coefficients. The differences are, however, too small to be of any practical significance in the present investigation, and the usual heterogeneity coefficient may be employed.

TABLE IV.—*Soil heterogeneity coefficients (correlations for all rows) and average correlations between the electrical resistance of soils from various levels in neighboring borings in the experimental field at Sacaton, Ariz., in 1921*

	Correlations for all rows		Average for pairs of rows
	$r \pm E_r$	r/E_r	
First foot	+0.550±0.053	10.460	0.6121
Second foot	+.349± .066	5.273	.4903
Third foot	+.517± .055	9.367	.5637
Fourth foot	+.408± .063	6.487	.4808
First to fourth foot	+.581± .050	11.649	.6824

(5) The coefficient for the average resistance of the first to fourth foot inclusive [9] is almost without exception higher than that for any individual foot. This may be due to the fact that experimental errors of measurement are to some extent ruled out when the average values of the upper 4 feet are taken. It may be in part due to differences in the extent to which the salts have been brought to the surface or washed down from the surface in various parts of the field, due to differences in the physical properties of the soil or to other factors, treated in detail by Scofield (*24*).

ANALYSIS OF DATA FOR THE EXPERIMENT OF 1922

The culture of 1922 comprised three varieties of cotton (Pima Egyptian, Meade Upland, and Acala Upland), which were planted in subrows of 10 feet each, each group of three such subrows constituting a "triplet," which is the unit of statistical analysis. The physicochemical constants for the plant-tissue fluids were based on samples drawn at random from the 20 feet (20 hills, as originally planted) of culture available from two adjoint rows, 1–2, 3–4, 5–6, . . . 23–24. The soil samples were based on three borings distributed between the pairs of rows over the 10 feet planted to each variety of cotton. Both soil and tissue-fluid constants are, therefore, reasonably typical

[8] Original coefficients in Table III; and average values in Table IV.

[9] There may be some question as to the legitimacy of averaging the resistances of different levels of soil in order to obtain some measure of the concentration of salts in the whole soil column. As this presents a special problem it will not be discussed here further than to say that it was not practicable in the case of the present investigation to use any other method.

of the conditions afforded by a cultural plot 10 feet in length and two rows in width.

In considering the heterogeneity of the field as expressed in the correlation between the electrical resistance of the soils, or of the plant-tissue fluid properties of associated plots, certain peculiarities of the planting scheme [10] must be considered.

Since the order of planting is

$$| A\ P\ M | A\ P\ M | A\ P\ M | \ldots | \ldots |$$

the soils of A (Acala) and P (Pima) and of P and M (Meade) of each triplet are immediately associated, while those of A and M are separated by one subplot of 10-foot length. If, however, the range of possible combinations be extended to include the cultures of an adjoining triplet, it will be possible to obtain Meade and Acala cotton in immediate association, i. e., on adjoining subplots. Thus it is possible to determine the correlations

$$r_{PA},\ r_{PM},\ r_{AM}$$

for either tissue fluids or for soil characteristics (conductivities) on the basis of immediately associated subplots. For r_{PA} and r_{PA} six combinations per pair of rows are available, or 72 for the field as a whole. For r_{AM}, which must involve the use of two adjacent triplets, only five combinations per row are available, or 60 for the field as a whole.

If the spatial range of subplots which may serve as the basis for correlation coefficients to include those of adjacent triplets be extended, correlations between Pima and Acala and Pima and Meade, in which the members of each pair are separated by one intervening subplot, may be determined. Such correlations will of necessity be based on frequencies of 60.

This course has the advantage of providing two series of correlations quite comparable with those for the plant-tissue fluids to be discussed later.

The coefficients appear in Table V, which is similar to Table III except for the fact that the constants are divided into two sections, the first of which contains the coefficients for immediately associated subplots, whereas the second contains those for subplots separated by an intervening subplot of 10 feet in length.

It is statistically impossible for all of these correlations to be based on the same number of plots. Two of the coefficients for immediately associated subplots, r_{PA}, r_{PM}, are based on subplots of the same triplet and represent 72 pairs of determinations, whereas one, r_{AM}, is based on the immediately contiguous subplot of adjoining triplets and involves only 60 pairs of determinations. Similarly, one of the correlations between subplots separated by an intervening area, $r_{A/M/}$, is based on determinations from the same triplet, and involves 72 pairs of determinations, whereas two, $r_{P/A/}$, $r_{P/M/}$, are based on determinations made on the subplots of adjoining triplets and involve only 60 pairs of readings each.

[10] The planting plan was drawn up primarily to make possible a crucial comparison between Pima Egyptian and Meade and Acala Upland cotton, and to test the relative influence of soil conditions on the characteristics of these three varieties. Had the experiment been planned solely as a means of investigating soil properties, it would have been arranged somewhat differently.

These coefficients are, with minor exceptions, substantial values. They fully confirm conclusions 1, 3, and 5 based on the data for 1921 above. They furnish no evidence on conclusion 4. Their bearing on 2 will now be considered.

On theoretical grounds, and as a result of the experience expressed in conclusion 2 based on the data of 1921, one might expect the closest correlations to be those between the most closely associated subplots. To test further the validity of this conclusion, the differences $r_{PA} - r_{P/A/}$, $r_{PM} - r_{P/M/}$, and $r_{AM} - r_{A/M/}$ have been determined. These are entered in the third section of constants of Table V.

TABLE V.—*Comparison of the correlation coefficients measuring the relationship between the soil salinity as expressed in terms of electrical resistance of the soil from different levels in subplots on which Pima Egyptian and Meade and Acala Upland cotton were grown, when the subplots are immediately adjacent and when they are separated by an intervening subplot.*

[Coefficients from the experiment of 1922]

	Second variable for subplots in immediate association		Second variable for subplots separated by an intervening plot		Difference in correlation when determined from immediately associated and from separated subplots	
	Resistance for subplot for Acala Upland cotton	Resistance for subplot for Meade Upland cotton	Resistance for subplot for Acala Upland cotton	Resistance for subplot for Meade Upland cotton	Difference for Acala Upland cotton	Difference for Meade Upland cotton
First variable, soil of subplot for Pima Egyptian cotton:						
Resistance of first foot, R_1	0.471±0.062	0.672±0.044	0.387±0.074	0.291±0.080	−0.084±0.097	−0.381±0.091
Resistance of second foot, R_2	.677± .043	.686± .042	.383± .074	.271± .081	−.294± .086	−.415± .091
Resistance of third foot, R_3	.699± .041	.678± .043	.434± .071	.194± .084	−.265± .082	−.484± .094
Resistance of fourth foot, R_4	.637± .047	.645± .047	.363± .076	.175± .084	−.274± .089	−.470± .096
Average of correlations	.621±	.670±	.392±	.233±	−.229±	−.437±
Average resistance, R_{1-4}	.714± .039	.747± .035	.486± .067	.258± .081	−.228± .078	−.489± .088
First variable, soil of subplot for Acala Upland cotton:						
Resistance of first foot, R_1		.494± .066		.290± .073		−.204± .098
Resistance of second foot, R_2		.601± .056		.573± .053		−.028± .077
Resistance of third foot, R_3		.559± .060		.359± .069		−.200± .091
Resistance of fourth foot, R_4		.549± .061		.470± .062		−.079± .087
Average of correlations		.551±		.423±		−.128±
Average resistance, R_{1-4}		.631± .052		.473± .062		−.158± .081

The differences are negative throughout, and in general may be considered statistically significant in comparison with their probable errors.[11] They show, therefore, that the correlations for subplots separated by an intervening subplot are materially lower than those for soils which are immediately contiguous.

[11] The probable errors of these differences have been computed by the usual formula for the probable error of a difference. It is possible that it is not strictly applicable under present conditions, but in view of the fact that the probable error would be lowered rather than raised by any corrective term no serious error can result.

THE CORRELATION BETWEEN THE CHARACTERS OF THE PLANTS GROWN ON ASSOCIATED SUBPLOTS

If the physical or chemical characteristics of the soil with respect to which the field is heterogeneous have an influence upon the plants grown upon it, a correlation between the characteristics of the plants of associated subplots should result.

That such correlations, whatever the underlying causes to which they may be due, have substantial values, has been demonstrated not merely for yield in a wide range of agricultural plants (*9*) but for freezing-point depression, specific electrical conductivity, ratio of specific electrical conductivity to freezing-point depression, and hydrogen-ion concentration in a series of experiments conducted on cotton in 1921 (*11*), and for chloride (*12*) and sulphate (*15*) content in subsequent cultures of Egyptian and Upland cotton.

TABLE VI.—*Comparison of the correlation coefficients measuring the relationship between the freezing-point depression, specific electrical conductivity, and chloride content of the leaf-tissue fluids of Pima Egyptian and of Meade and Acala Upland cotton, when the cultures are grown upon immediately associated subplots and upon plots which are separated by an intervening subplot*

[Coefficients from the experiment of 1922]

	Subplots in immediate association		Subplots separated by an intervening plot		Difference in correlation when determined from immediately associated and from separated plots	
	Second variable, Acala Upland cotton	Second variable, Meade Upland cotton	Second variable, Acala Upland cotton	Second variable, Meade Upland cotton	Difference for Acala Upland cotton	Difference for Meade Upland cotton
First variable, Pima Egyptian cotton:						
Freezing-point depression, Δ:						
First series	0.693±0.041	0.756±0.034	0.269±0.081	0.399±0.074	−0.424±0.091	−0.357±0.081
Second series	.797± .029	.827± .026	.663± .051	.422± .074	−.134± .059	−.405± .078
Specific electrical conductivity, κ:						
First series	.436± .065	.516± .059	.145± .086	.199± .085	−.291± .108	−.317± .103
Second series	.681± .043	.674± .044	.564± .060	.394± .075	−.117± .074	−.280± .087
Ratio, conductivity to depression, κ/Δ:						
First series	.399± .067	.565± .055	.343± .077	.094± .088	−.056± .102	−.471± .104
Second series	.494± .061	.361± .071	.168± .088	.144± .089	−.326± .107	−.247± .114
Chloride content, *Cl*:						
First series	.684± .043	.670± .045	.413± .074	.300± .082	−.271± .086	−.370± .094
Second series	.603± .052	.603± .052	.341± .079	.291± .083	−.262± .095	−.312± .098
First variable, Acala Upland cotton:						
Freezing-point depression, Δ:						
First series		.520± .064		.602± .051		+.082± .082
Second series		.765± .037		.674± .044		−.091± .057
Specific electrical conductivity, κ:						
First series		.234± .083		.391± .068		+.158± .107
Second series		.691± .046		.531± .058		−.160± .074
Ratio, conductivity to depression, κ/Δ:						
First series		.094± .087		.537± .057		+.443± .104
Second series		.260± .084		.319± .073		+.059± .111
Chloride content, *Cl*:						
First series		.449± .072		.441± .065		−.008± .097
Second series		.538± .064		.324± .073		−.214± .097

In the present series of data there is the advantage of comparing correlations deduced from measurements on the tissue-fluid properties of plants growing on immediately associated subplots and on plots separated by an intervening area. The coefficients and their probable errors appear in Table VI constructed in the same way as Table V.

The correlations for osmotic concentration in terms of freezing-point depression range from +0.520 to +0.827 for the plants of subplots which are in immediate association, and from +0.269 to +0.674 for the cultures separated by an intervening area of the same size. Thus the correlations for immediately associated cultures are of the same order of magnitude as those demonstrated for cultures of Pima Egyptian and Meade and Acala Upland cotton made in 1921 (*11, Table X*). The coefficients for plants separated by an intervening culture are, in general, somewhat lower, but there is one case in which the correlations for the separated subplots is insignificantly higher than that for the immediately contiguous cultures.

The constants for specific electrical conductivity are somewhat irregular in magnitude, but they are of the general order of magnitude found for this variable in earlier investigations on these three varieties of cotton (*11, Table XVI*). They are without exception smaller than those for the homologous constants for freezing-point depression. This is in accord with the results found in 1921 (*11, Table XXIV*). The coefficients for the subplots which are separated by an intervening subplot are in three cases smaller and in one case insignificantly larger than the constants for those which are in immediate association.

The coefficients measuring the correlation between the ratios of specific electrical conductivity to freezing-point depression, κ/Δ, in the tissue fluids of associated plots are of the general order of magnitude and irregularity found in earlier investigations (*11, Table XVII*). In three of the four cases the coefficients for slightly separated subplots are numerically lower than those for immediately adjacent cultures.

The results for this large independent experiment therefore fully confirm those obtained in the first extensive investigation (*11*), and extend them by showing that, in general, the tissue fluids of plants produced on somewhat separated subplots are less closely correlated than those of plants grown on immediately contiguous areas.

These results show clearly that there is a general similarity between the correlations for the properties of the soils and the correlations for the characteristics of the plants which they produce. That this similarity represents a likeness of trend of homologous values rather than a strictly quantitative parallelism is evident from a closer study of the two tables. This relationship between the coefficients for the plant-tissue fluids and those for the soils upon which the organisms were produced presents a complex problem, which is now under investigation. The quantitative discrepancies between the two series of homologous coefficients—one for the soils and the other for the plants produced upon them—can not invalidate the general conclusion that there is such an intimate relationship between the properties of the soils and the characteristics of the plants produced on an agricultural field, that with proper experi-

mental and statistical technique many of the problems of the relationship between the soil and the plant can be investigated under the most ideal conditions for agronomic research—those of plot or field cultures in which the crop plant may be grown without the artificiality of complex laboratory control and subject only to the conditions which surround its normal commercial production.

The following sections are devoted to the establishment of this conclusion by an appeal to the direct correlations between the properties of the soil and the characteristics of the plant.

THE RELATIONSHIP BETWEEN THE ELECTRICAL RESISTANCE OF THE SOIL AND THE PROPERTIES OF THE PLANT-TISSUE FLUIDS

Because of interesting water relations (*22*), because of its tolerance :a relatively high soil salinity (as noted long ago by Kearney and Means (*16*), by Kearney (*17*), and by Balls (*1*)), and because of the high osmotic concentration, specific electrical conductivity (*11*), chloride (*13*, *12*), and sulphate content (*15*), demonstrated in the course of the series of investigations to which this belongs—the cotton plant (especially the Egyptian type) should be a suitable subject for the investigation of the relationship between the concentration of the soil solution in soils of relatively high salinity and the characteristics of the plants. The results of the present investigation fully justify this presumption.

While the most logical arrangement of the materials might be that in which the relationship between the resistance of the soil and the electrical conductivity of the plant-tissue fluids is discussed first, it is more convenient to treat the osmotic concentration of the plant-tissue fluids at the outset.

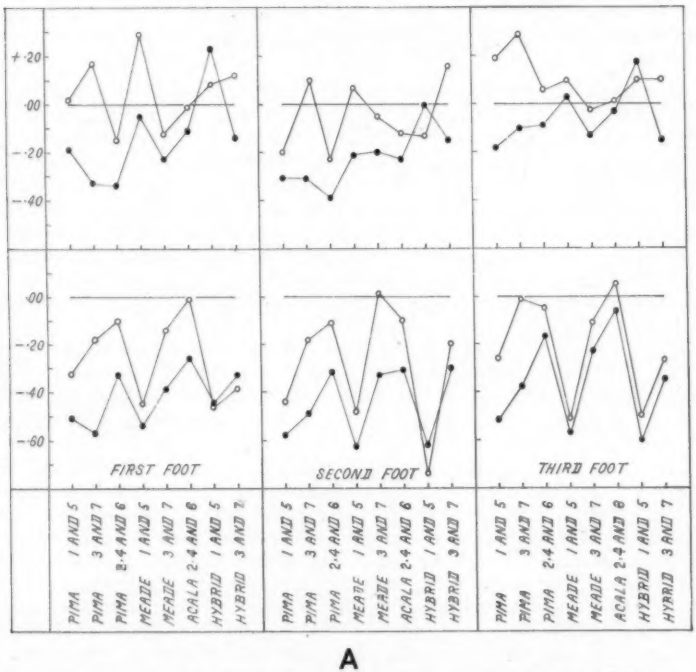
The correlation between the resistance of the soil solution and the osmotic concentration of the plant-tissue fluids furnishes a measure of the relationship between the concentration of the ionized conducting electrolytes of the soil and the concentration of all solutes (both ionized and nonionized electrolytes and nonelectrolytes) of the leaf fluids. The correlation between the electrical resistance of the soil solution and the specific electrical conductivity of the plant-tissue fluids furnishes some measure of the concentration of dissociated (conducting) electrolytes in the inorganic and biological systems. A comparison of these correlations with those involving the total solutes of plant-tissue fluids will be drawn.

The determination of the correlation between the resistance of the soil solution and the chloride content of the plant-tissue fluids makes possible the consideration of the relationship between all of the ionized solutes and the concentration of a specific ion, the chlorine ion,[12] in the plant-tissue fluids.

It is desirable to consider these interrelationships not merely in the universally comparable terms of the correlation coefficient, but also in terms of the regression of the tissue-fluid properties on the soil resistances. For this purpose not merely the regression equations, but, as a basis of comparison, the empirical means of the freezing-point depression, specific electrical conductivity, and chloride content for each grade of soil resistance, are required.

[12] This statement does not imply that the chlorides are wholly ionized.

By the use of the proper formulae (*3*), the product moment may be calculated from the frequency of the various grades of soil resistance and the mean values of the plant-tissue fluid characters which are required for a graphical representation of the relationship between the soil and the plant variable. Limitation of space precludes the publication of all of the data, but Tables VII and VIII, showing the relationship between the soil resistance of the third and fourth foot of soil and the tissue-fluid properties in Pima cotton, will serve as



A

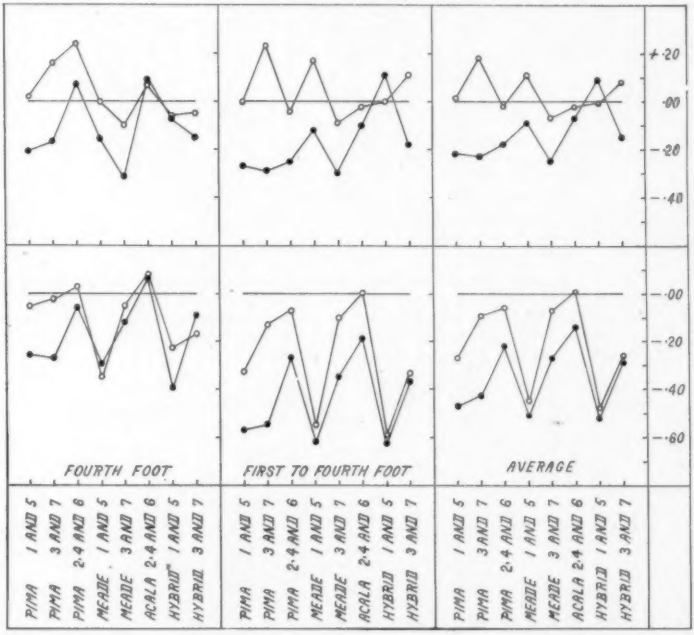
FIG. 1.—A. Magnitudes of correlation between the electrical resistance of the soil and freezing-point depression Δ (solid dots) and specific electrical conductivity κ (circles) of leaf-tissue fluids in Pima Egyptian and Meade and Acala Upland cotton, and in their F_1 hybrid, as grown at Sacaton, Ariz., in 1921. Lower figures represent the first series of determinations and the upper figures the second series. (See fig. 1, B, for results for fourth foot of soil, for first to fourth foot, and for average correlation.)

illustrations. In these tables the soil resistance is treated in units of 25 ohms range. The frequencies, F, of these resistances are limited by the number of determinations available for Δ, K, and Cl in the first or second series of determinations, as indicated by the subscripts. Taken in connection with Tables I and II, which give the resistances of the first (R_1) and of the second (R_2) foot of soil for two of the sub-

plots, these tables give a general indication of the order of soil resistances involved.

The frequency distribution and the statistical constants for the tissue-fluid properties will be treated in connection with a different problem in another paper.

In presenting the data each foot of soil is first treated separately, thus lessening the possibility of arithmetical errors and providing some means of estimating the significance of different (arbitrarily



B

FIG. 1.—B. Magnitudes of correlation between electrical resistance of the soil and freezing-point depression Δ (solid dots) and specific electrical conductivity κ (circles), of leaf-tissue fluids in Pima Egyptian and Meade and Acala Upland cotton, and in their F_1 hybrid, as grown at Sacaton, Ariz., in 1921. Lower figures represent the first series of determinations, and the upper figures the second series. (See fig. 1, A, for results for the first, second, and third foot of soil.)

defined) layers of the soil in determining the characters of the plant. As a more trustworthy measure of the soil as a whole, the correlation between the average resistance of the four upper feet and the characteristics of the plants produced will be considered, as well as the average value of the four correlations for individual layers (first to fourth foot) of soil.

TABLE VII.—*Average values of freezing-point depression, specific electrical conductivity, and chloride content in a first and second series of determinations on the leaf-tissue fluids of Pima Egyptian cotton grown on subplots of different salinities as measured in terms of the electrical resistance in ohms of the third foot of soil of the field at Sacaton, Ariz., in the experiment of 1922*

Soil resistance, third foot	Mean freezing-point depression, Δ				Mean specific electrical conductivity, κ				Mean chloride content, Cl			
	F_1	Δ_1	F_2	Δ_2	F_1	κ_1	F_2	κ_2	F_1	Cl_1	F_2	Cl_2
50	14	1.423	14	1.485	14	0.03307	14	0.03300	14	5.714	12	5.750
75	15	1.364	14	1.457	15	.03280	14	.03207	14	5.000	14	5.750
100	10	1.320	9	1.408	9	.03166	10	.03140	10	4.525	10	5.400
125	10	1.267	10	1.311	10	.03140	10	.02990	9	4.333	10	4.900
150	2	1.302	2	1.395	2	.03150	2	.03200	2	4.000	2	5.625
175	3	1.240	3	1.240	3	.03133	3	.02833	3	4.500	3	5.000
200	1	1.302	1	1.302	1	.02900	1	.02900	1	4.250	1	4.750
225	8	1.232	8	1.259	8	.03087	8	.02862	8	4.375	8	4.843
250	2	1.224	2	1.255	2	.02900	2	.03050	2	4.375	2	4.875
275	2	1.178	2	1.193	2	.03000	2	.03000	2	4.000	2	4.125
300	1	1.209	1	1.271	1	.03100	1	.03000	1	3.750	1	4.500
325	1	1.147	1	1.147	1	.02800	1	.02700	1	3.750	1	4.000
350	1	1.240	1	1.271	1	.03300	1	.03000	1	3.250	1	3.750
375												
400	1	1.240	1	1.209	1	.03200	1	.02700	1	3.500	1	3.500

TABLE VIII.—*Average values of freezing-point depression, specific electrical conductivity, and chloride content in a first and second series of determinations on the leaf-tissue fluids of Pima Egyptian cotton grown on subplots of different salinities as measured in terms of the electrical resistance in ohms of the fourth foot of soil of the field at Sacaton, Ariz., in the experiment of 1922*

Soil resistance, fourth foot	Mean freezing-point depression, Δ				Mean specific electrical conductivity, K				Mean chloride content, Cl			
	F_1	Δ_1	F_2	Δ_2	F_1	K_1	F_2	K_2	F_1	Cl_1	F_2	Cl_2
50	23	1.382	22	1.473	22	0.03254	23	0.03247	21	5.190	22	5.693
75	15	1.335	14	1.408	15	.03233	14	.03200	15	4.966	13	5.423
100	10	1.308	10	1.326	10	.03200	10	.03030	10	4.425	10	5.125
125	6	1.265	6	1.312	6	.03150	6	.02866	6	4.500	6	5.250
150	8	1.212	8	1.232	8	.03037	8	.02862	8	4.312	8	4.593
175	4	1.247	4	1.302	4	.03100	4	.03050	4	4.562	4	4.937
200	1	1.302	1	1.302	1	.02900	1	.02900	1	4.250	1	4.750
225	2	1.224	2	1.240	2	.02950	2	.02800	2	3.625	2	4.000
250	1	1.240	1	1.271	1	.03300	1	.03000	1	3.250	1	3.750
275	1	1.209	1	1.271	1	.03100	1	.03000	1	3.750	1	4.500

THE RELATIONSHIP BETWEEN THE ELECTRICAL RESISTANCE OF THE SOIL AND THE FREEZING-POINT DEPRESSION OF THE PLANT-TISSUE FLUIDS

The results for the relationship between the electrical resistance of the soil mass and the depression of the freezing point for the leaf-tissue fluids of the Egyptian, Upland, and F_1 hybrid plants grown in 1921 are set forth for the first and for the second series of determinations [13] in Table IX. They are also represented graphically by the position of the solid dots connected by solid lines on the scale of ordinates in the two parts of Figure 1.

[13] Unfortunately, soil borings were made in rows 1, 3, 5, and 7 only. In the absence of data for rows 2, 4, and 6 the measurements made in the contiguous rows may be utilized. Thus, for the collections of Pima and Acala cotton made in the second, fourth, and sixth rows, the soil determinations made in the first and third rows, third and fifth rows, and in the fifth and seventh rows may be utilized. In doing this the product moment for the correlation between the plants in the second row and the soil constants in both the first and third rows, for the plants in the fourth row and the soil constants in the third and fifth rows, and for the plants in the sixth row and the soil constants in the fifth and seventh rows is determined. Thus, the plants are weighted with the number of soil borings on either side. Since one doublet is missing, there is a total of 59 determinations on sap properties, or a weighted number of 118 for both plant and soil determinations for rows 2, 4, and 6.

TABLE IX.—*Correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression of the leaf-tissue fluids in Egyptian and Upland cotton and in their F_1 hybrid as grown at Sacaton, Ariz., in 1921*

	N	First foot		Second foot		Third foot		Fourth foot		All layers		Average correlations for all layers
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	
Pima Egyptian, from self-fertilized seed, rows, 1, 5:												
First series	39	−0.5124±0.0796	6.40	−0.5781±0.0719	8.02	−0.5210±0.0786	6.59	−0.2632±0.1005	2.60	−0.5704±0.0728	7.80	−0.4687
Second series	39	−.1853±.1042	1.77	−.3056±.0979	3.12	−.1826±.1043	1.75	−.2118±.1031	2.05	−.2698±.1001	2.70	−.2213
Difference		+.3271±.1311	2.50	+.2725±.1215	2.24	+.3384±.1306	2.59	+.0514±.1440	.36	+.3006±.1238	2.43	+.2474
Pima Egyptian, from bulk seed, rows 3, 7:												
First series	38	−.5656±.0744	7.64	−.4915±.0829	5.92	−.3803±.0935	4.04	−.2701±.1014	2.67	−.5487±.0764	7.22	−.4269
Second series	38	−.3293±.0975	3.35	−.3151±.0985	3.18	−.1017±.1082	.94	−.1673±.1063	1.57	−.2913±.1001	2.91	−.2284
Difference		+.2363±.1226	1.93	+.1764±.1287	1.37	+.2786±.1430	1.95	+.1028±.1469	.70	+.2574±.1259	2.04	+.1985
Pima Egyptian, from bulk seed, rows 2, 4, 6:												
First series	59	−.3345±.0551	6.06	−.3236±.0555	5.78	−.1738±.0602	2.90	−.0635±.0618	1.03	−.2657±.0577	4.58	−.2239
Second series	59	−.3352±.0551	6.09	−.3910±.0525	7.37	−.0890±.0615	1.43	+.0097±.0617	1.12	−.2454±.0583	4.22	−.1864
Difference		−.0007±.0779	.01	−.0674±.0764	.88	+.0848±.0861	.98	+.1332±.0873	1.53	+.0203±.0820	.25	+.0375
Meade Upland, from self-fertilized seed, rows 1, 5:												
First series	39	−.5420±.0762	7.13	−.6260±.0656	9.48	−.5671±.0732	7.76	−.3022±.0981	3.08	−.6193±.0665	9.23	−.5093
Second series	39	−.0454±.1077	.41	−.2091±.1032	2.02	+.0308±.1078	.28	−.1620±.1051	1.54	−.1202±.1064	1.13	−.0964
Difference		+.4966±.1319	3.76	+.4169±.1223	3.41	+.5979±.1303	4.59	+.1402±.1438	.97	+.4991±.1255	3.98	+.4129
Meade Upland, from bulk seed, rows 3, 7:												
First series	38	−.3929±.0925	4.22	−.3536±.0957	3.68	−.2289±.1038	2.17	−.1172±.1079	1.08	−.3553±.0956	3.69	−.2724
Second series	38	−.2314±.1035	2.22	−.2015±.1049	1.92	−.2325±.1035	2.24	−.3158±.0985	3.19	−.3003±.0995	3.00	−.2453
Difference		+.1615±.1388	1.16	+.1521±.1420	1.07	−.0066±.1466	.05	−.1986±.1461	1.36	+.0550±.1380	.40	+.0271
Acala Upland, from bulk seed, rows 2, 4, 6:												
First series	59	−.2612±.0578	4.51	−.3104±.0561	5.53	−.0603±.0618	.96	+.0618±.0618	1.00	−.1877±.0599	3.13	−.1425
Second series	59	−.1118±.0613	1.83	−.2342±.0586	3.96	−.0363±.0620	.58	+.0845±.0616	1.37	−.0972±.0615	1.56	−.0745
Difference		+.1494±.0843	1.77	+.0762±.0811	.94	+.0240±.0875	.27	+.0227±.0873	.26	+.0905±.0859	1.05	+.0680
F_1 Hybrid, rows 1, 5:												
First series	39	−.4557±.0855	5.30	−.6247±.0658	9.46	−.6006±.0690	8.71	−.3877±.0917	4.21	−.6246±.0658	9.46	−.5172
Second series	39	+.2350±.1020	2.30	−.0014±.1079	.01	+.1836±.1043	1.76	−.0652±.1075	.60	+.1101±.1066	1.02	+.0880
Difference		+.6907±.1331	5.19	+.6233±.1264	4.93	+.7842±.1251	6.27	+.3225±.1413	2.28	+.7347±.1253	5.86	+.6052
F_1 Hybrid, rows 3, 7:												
First series	38	−.3375±.0969	3.48	−.3994±.0919	4.33	−.3497±.0960	3.64	−.0912±.1085	.83	−.3748±.0940	3.98	−.2945
Second series	38	−.1407±.1072	1.31	−.1530±.1068	1.42	−.1534±.1068	1.42	−.1489±.1060	1.39	−.1846±.1056	1.74	−.1490
Difference		+.1968±.1445	1.36	+.2464±.1409	1.75	+.1963±.1436	1.37	−.0577±.1523	.38	+.1902 ±.1414	1.35	+.1455

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Limiting attention for the moment to the constants for the first series of determinations (that made August 6 to 16, inclusive), it may be noted that with one exception (the relationship for the fourth foot in Acala Upland grown from bulk seed) the constants show a negative correlation between soil resistance and the freezing-point depression of the tissue fluids. Since soil readings are given in terms

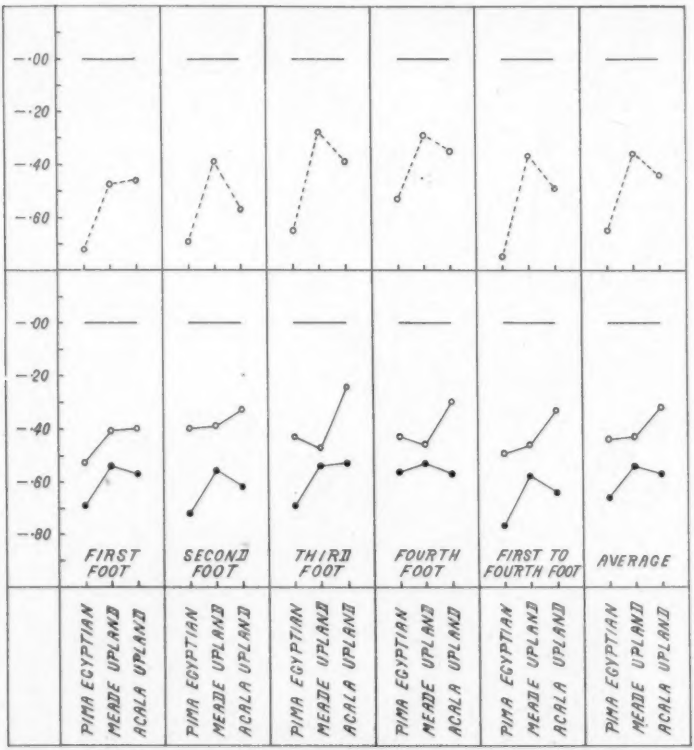


FIG. 2.—Magnitudes of correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression Δ (solid dots connected by solid lines), specific electrical conductivity κ (circles connected by solid lines), and chloride content Cl (circles connected by broken lines), in Pima Egyptian and in Meade and Acala Upland cotton for the first series of determinations made at Sacaton, Ariz., in 1922. (See fig. 3 for results for second series of determinations.)

of resistance, a negative correlation indicates that a higher electrolyte content of the soil is accompanied by a higher solute content of the plant-tissue fluids. The coefficients are of substantial numerical magnitude and, individually considered, are practically without exception significant in comparison with their probable errors.

Turning to the correlations between the second series of determinations on sap properties and the soil resistances, it is found that, with 6 exceptions, the 40 coefficients (Table IX and fig. 1, A) are negative in sign. While most of them would be considered insignificant in comparison with their probable errors, the general average

of the correlations for the 4 feet are in seven of the eight cases negative in sign. Thus the results for the second series tend to confirm those for the first series in indicating some relationship between the concentration of the soil solution and the concentration of the plant-tissue fluids. The relationship is, however, more intimate in the first series of tissue-fluid determinations than in the second.

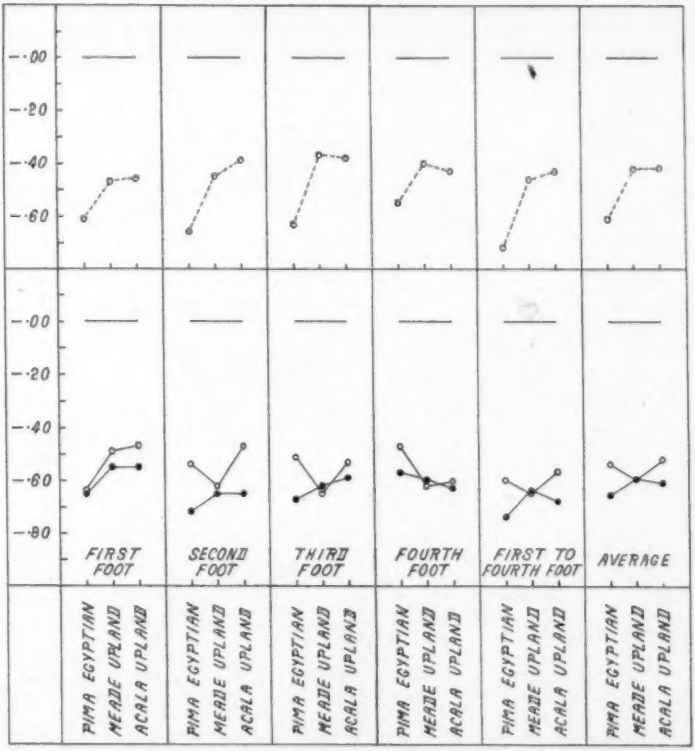


FIG. 3.—Magnitudes of correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression Δ (solid dots connected by solid lines), specific electrical conductivity κ (circles connected by solid lines), and chloride content *Cl* (circles connected by broken lines), in Pima Egyptian and in Meade and Acala Upland cotton for the second series of determinations made at Sacaton, Ariz., in 1922. (See fig. 2 for results for first series of determinations.)

This is clearly shown by the differences between the correlations for the first and second series of determinations. These differences have been taken (constant for second series) minus (constant for first series), with due regard to signs. As the correlations in both series of determinations are generally negative, a positive sign in the difference indicates numerically a larger negative correlation in the first series of determinations.[14]

[14] It has been necessary to calculate the probable errors of the differences between these correlations without regard to the possible correlation between the two correlation coefficients. Since such correlation would tend to reduce the magnitudes of the probable errors of the differences, no arguments of the present discussion are invalidated by the neglect of this factor.

In both first and second series of determinations the correlation between the average resistance of the four upper feet of soil and the osmotic concentration of the plant-tissue fluids is intermediate in value between the highest and lowest coefficients for the individual foot layers. The coefficient for the entire soil column (as nearly as this can be approximated by taking the average resistance of the four individual feet) is, however, in every instance higher than the average values of the four individual coefficients for the foot samples.

This result is what one might expect. The cotton plant feeds, to some extent at least, at various depths. The osmotic concentration

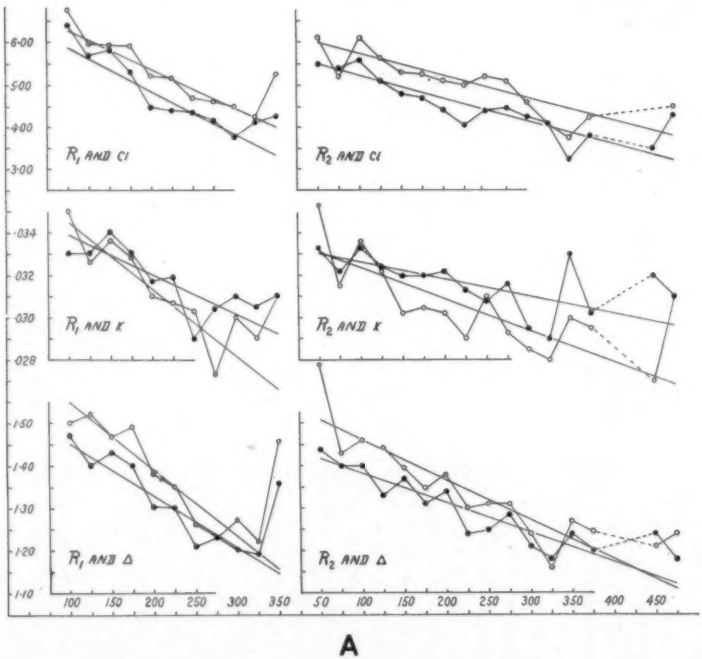


FIG. 4.—A. Regression straight lines and empirical means showing change in freezing-point depression Δ, in specific electrical conductivity κ, and in chloride content *Cl*, associated with variation in the electrical resistance (in ohms) of the soil of the first foot R_1, and of the second foot R_2, in Pima Egyptian cotton, as grown at Sacaton, Ariz., in 1922. Solid dots represent the first series of determinations and circles represent the second series. (For third foot and fourth foot, see fig. 4, B.)

should therefore be influenced by the solutes throughout the entire range of actual root penetration. Furthermore, the movement of salts in the soil (*24*) is presumably such that the condition as measured on a given day is not necessarily what it was at the time the chief absorption by the plant took place. Finally, errors of measurement of the conductivities of the individual samples are eliminated to some extent when the average of the four determinations is taken.

All these factors would tend to make the correlation between the average resistance of the four upper feet of soil and the plant-tissue fluids more intimate than that for the individual soil layers.

Turning now to the results for the larger series of determinations made in 1922, the constants for the first series are shown in Table X and are represented by the solid dots connected by the firm lines in Figure 2, while those for the second series are shown in Figure 3. The correlations between the soil resistance and osmotic concentration, as expressed in terms of freezing-point depression, are all negative and of a substantial order of magnitude.

The coefficients are from about 9 to about 24 times as large as their probable errors. It is clear that for Pima Egyptian and Meade and Acala Upland cotton there is an intimate and statistically sig-

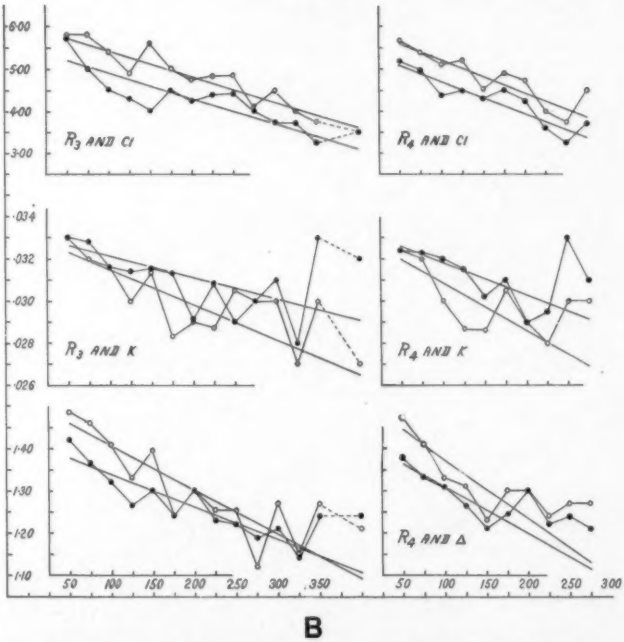


FIG. 4.—B. Regression straight lines and empirical means showing change in freezing-point depression Δ, in specific electrical conductivity κ, and in chloride content Cl, associated with variation in the electrical resistance (in ohms) of the soil of the third foot R_3, and of the fourth foot R_4, in Pima Egyptian cotton as grown at Sacaton, Ariz., in 1922. Solid dots represent the first series of determinations and circles represent the second series. (For first foot and second foot see fig. 4, A.)

nificant correlation between the electrical resistance of the saturated soil mass and the osmotic concentration of the leaf-tissue fluids of plants growing upon it.

The coefficients for the upper 3 feet of soil are above −0.60 in both series of determinations on Pima Egyptian cotton, and above −0.50 for Meade and Acala Upland cotton. The coefficients are perhaps slightly lower for the fourth foot of soil than for the upper 3 feet, in both series. The correlations for the average resistance of the upper 4 feet of soil are of the order −0.75 for the Pima Egyptian and −0.60 to −0.70 for the Upland varieties. The correlations for

Eygptian cotton seem to be slightly higher than for the Upland varieties of cotton, at least for the upper 3 feet of soil.

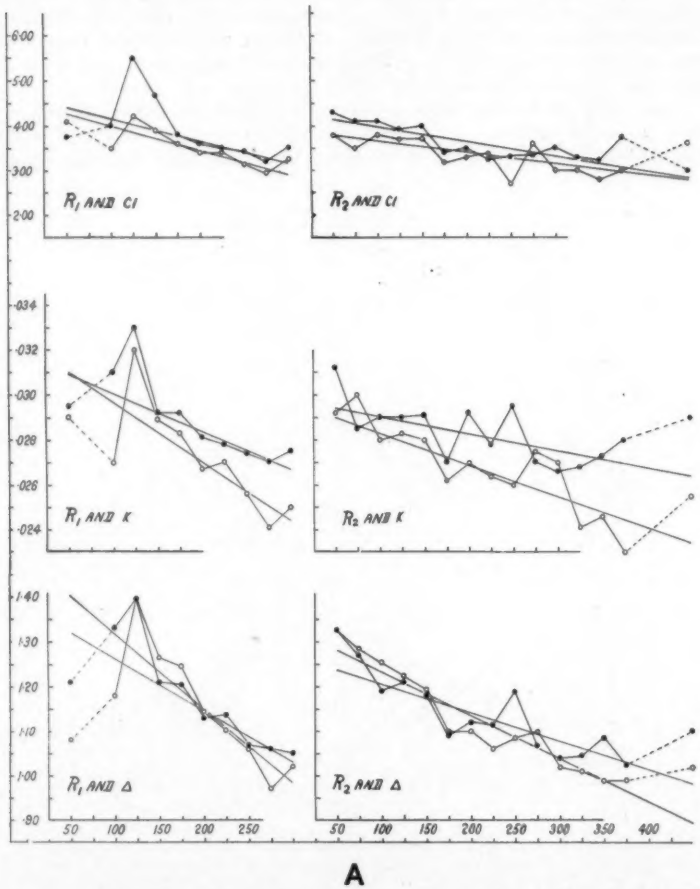


FIG. 5.—A. Regression straight lines and empirical means showing change in freezing-point depression Δ, in specific electrical conductivity κ, and in chloride content *Cl*, associated with variation in the electrical resistance (in ohms) of the soil of the first foot R_1, and of the second foot R_2, in Acala Upland cotton as grown at Sacaton, Ariz., in 1922. Solid dots represent the first series of determinations and circles represent the second series. (For third foot and fourth foot, see fig. 5, B.)

A study of the coefficients for the determinations made in 1921 suggests that there are considerable differences between the correlations for the individual layers of soil. This might be due to differences in root penetration in the several soil layers, or to differences in the extent to which water and its solutes are drawn by the plant from the various levels of the soil. The results for the series of 1922 show no such conspicuous differences of this kind as are found in the

first series for 1921, and if there be such differences in correlation they can be established only by further investigation.

The equations to the straight lines showing the change in the mean value of freezing-point depression, Δ, associated with variation in soil resistance appear in Table XI, which also contains the results

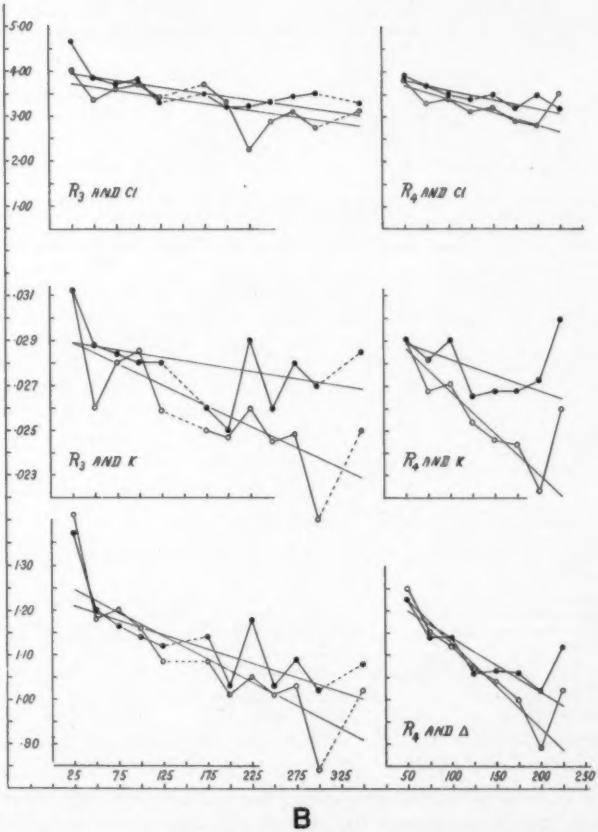


FIG. 5.—B. Regression straight lines and empirical means showing change in freezing-point depression Δ, in specific electrical conductivity κ, and in chloride content *Cl*, associated with variation in the electrical resistance (in ohms) of the soil of the third foot R_3, and of the fourth foot R_4, in Acala Upland cotton, as grown at Sacaton, Ariz., in 1922. Solid dots represent the first series of determinations and circles represent the second series. (For first and second foot see fig. 5, A.)

for specific electrical conductivity, κ, and for chloride content, *Cl*, to be discussed later. The equations are given by

$$\Delta = \left(\bar{\Delta} - r_{R\Delta}\frac{\sigma_\Delta}{\sigma_R}\bar{R}\right) + r_{R\Delta}\frac{\sigma\Delta}{\sigma_R}R$$

where r has its usual significance, the bars denote means, and the sigmas denote the standard deviations of freezing-point depression, Δ,

or soil resistance, R. This relationship is shown graphically in terms of the empirical and theoretical means of osmotic concentration in plants grown on various soils on Figure 4 for Pima Egyptian, Figure 5 for Meade Upland, and Figure 6 for Acala Upland cotton.

TABLE X.—*Correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression of the leaf-tissue fluids in Pima Egyptian and in Meade and Acala Upland cotton as grown at Sacaton, Ariz., in 1922*

	N	First foot		Second foot		Third foot	
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/Er
Pima Egyptian:							
First series	71	−0.6921±0.0417	16.60	−0.7176±0.0388	18.49	−0.6857±0.0424	16.17
Second series	69	−.6549± .0463	14.12	−.7165± .0395	18.13	−.6763± .0440	15.35
Difference		+.0372± .0623	0.60	+.0011± .0554	0.02	+.0094± .0611	0.15
Meade Upland:							
First series	71	−.5352± .0571	9.37	−.5638± .0546	10.33	−.5352± .0571	9.37
Second series	69	−.5498± .0566	9.71	−.6478± .0471	13.75	−.6166± .0503	12.26
Difference		−.0146± .0804	0.18	−.0840± .0721	1.17	−.0814± .0761	1.07
Acala Upland:							
First series	71	−.5734± .0537	10.67	−.6159± .0496	12.40	−.5313± .0574	9.25
Second series	69	−.5513± .0565	9.75	−.6510± .0467	13.92	−.5973± .0522	11.44
Difference		+.0221± .0779	0.28	−.0351± .0681	0.52	−.0660± .0776	0.85

	Fourth foot		All layers		Average correlations for all layers
	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	
Pima Egyptian:					
First series	−0.5667±0.0543	10.43	−0.7748±0.0319	24.22	−0.6655
Second series	−.5732± .0545	10.51	−.7481± .0357	20.93	−.6552
Difference	−.0065± .0769	0.08	+.0267± .0479	0.56	+.0103
Meade Upland:					
First series	−.5312± .0574	9.25	−.5788± .0532	10.87	−.5414
Second series	−.5991± .0520	11.51	−.6485± .0470	13.78	−.6033
Difference	−.0679± .0775	0.88	−.0697± .0710	0.98	−.0619
Acala Upland:					
First series	−.5739± .0536	10.69	−.6392± .0473	13.50	−.5736
Second series	−.6303± .0489	12.88	−.6800± .0436	15.58	−.6075
Difference	−.0564± .0726	0.78	−.0408± .0643	0.63	−.0339

The mean values of freezing-point depression are distributed with reasonable regularity about the theoretical lines. In evaluating the goodness of fit of these lines one must remember that the total number of pairs of determinations on soil and plant does not in any case exceed 72. In view of the fact that the range of variation in soil resistance is wide, many of the "means" are necessarily based on only a single determination in the two systems. The means of osmotic concentration are, therefore, greatly influenced by the probable errors of random sampling. In view of the facts just mentioned, the trend of the means is such as to justify the acceptance of the straight-line equation as a reasonable graduation of the results, at least until further data are available. A more crucial mathematical test of the goodness of fit of the equations must wait until larger series are available.

TABLE XI.—*Equations to the straight lines showing the changes in the mean freezing-point depression, mean specific electrical conductivity, and mean chloride content of the leaf-tissue fluids in Pima Egyptian and in Meade and Acala Upland cotton, associated with variation in the salinity of the soil as measured in terms of electrical resistance in ohms at various levels in the field at Sacaton, Ariz., experiment of 1922*

Series of plants and layers of soil	Regression of freezing-point depression, Δ, on soil resistance, R	Regression of specific electrical conductivity, κ, on soil resistance, R	Regression of chloride content, $Cl.$ on soil resistance, R
Pima Egyptian, first series:			
First foot	$\Delta = 1.57683 - 0.001226\ R$	$\kappa = 0.035907 - 0.0000190\ R$	$Cl. = 6.9213 - 0.01031\ R$
Second foot	$\Delta = 1.45295 - .000690\ R$	$\kappa = .033440 - .0000079\ R$	$Cl. = 5.8081 - .00546\ R$
Third foot	$\Delta = 1.41935 - .000790\ R$	$\kappa = .033177 - .0000101\ R$	$Cl. = 5.5187 - .00600\ R$
Fourth foot	$\Delta = 1.41562 - .001000\ R$	$\kappa = .033411 - .0000155\ R$	$Cl. = 5.4972 - .00761\ R$
Pima Egyptian, second series:			
First foot	$\Delta = 1.70860 - .001579\ R$	$\kappa = .037680 - .0000316\ R$	$Cl. = 7.2074 - .00915\ R$
Second foot	$\Delta = 1.55896 - .000937\ R$	$\kappa = .033880 - .0000147\ R$	$Cl. = 6.2930 - .00523\ R$
Third foot	$\Delta = 1.51329 - .001064\ R$	$\kappa = .033139 - .0000166\ R$	$Cl. = 6.0484 - .00604\ R$
Fourth foot	$\Delta = 1.51235 - .001387\ R$	$\kappa = .033258 - .0000230\ R$	$Cl. = 6.0412 - .00794\ R$
Meade Upland, first series:			
First foot	$\Delta = 1.45446 - .001093\ R$	$\kappa = .033897 - .0000177\ R$	$Cl. = 4.4664 - .00629\ R$
Second foot	$\Delta = 1.34990 - .000646\ R$	$\kappa = .031972 - .0000094\ R$	$Cl. = 3.7211 - .00299\ R$
Third foot	$\Delta = 1.32590 - .000831\ R$	$\kappa = .032014 - .0000152\ R$	$Cl. = 3.5106 - .00301\ R$
Fourth foot	$\Delta = 1.32821 - .001140\ R$	$\kappa = .032034 - .0000206\ R$	$Cl. = 3.3118 - .00182\ R$
Meade Upland, second series:			
First foot	$\Delta = 1.53629 - .001547\ R$	$\kappa = .033779 - .0000260\ R$	$Cl. = 3.9366 - .00538\ R$
Second foot	$\Delta = 1.41108 - .001028\ R$	$\kappa = .031930 - .0000186\ R$	$Cl. = 3.3809 - .00298\ R$
Third foot	$\Delta = 1.37471 - .001332\ R$	$\kappa = .031577 - .0000265\ R$	$Cl. = 3.2116 - .00335\ R$
Fourth foot	$\Delta = 1.37318 - .001777\ R$	$\kappa = .031428 - .0000344\ R$	$Cl. = 3.2433 - .00492\ R$
Acala Upland, first series:			
First foot	$\Delta = 1.37900 - .001138\ R$	$\kappa = .031784 - .0000168\ R$	$Cl. = 4.7115 - .00503\ R$
Second foot	$\Delta = 1.27171 - .000641\ R$	$\kappa = .029811 - .0000075\ R$	$Cl. = 4.3277 - .00326\ R$
Third foot	$\Delta = 1.22983 - .000647\ R$	$\kappa = .029139 - .0000062\ R$	$Cl. = 4.0295 - .00271\ R$
Fourth foot	$\Delta = 1.26349 - .001239\ R$	$\kappa = .029638 - .0000137\ R$	$Cl. = 4.0713 - .00410\ R$
Acala Upland, second series:			
First foot	$\Delta = 1.48685 - .001675\ R$	$\kappa = .032455 - .0000266\ R$	$Cl. = 4.5696 - .00545\ R$
Second foot	$\Delta = 1.32982 - .000968\ R$	$\kappa = .029717 - .0000139\ R$	$Cl. = 3.9242 - .00241\ R$
Third foot	$\Delta = 1.27520 - .001040\ R$	$\kappa = .029348 - .0000182\ R$	$Cl. = 3.8166 - .00281\ R$
Fourth foot	$\Delta = 1.32619 - .001951\ R$	$\kappa = .030513 - .0000371\ R$	$Cl. = 3.9836 - .00560\ R$

THE RELATIONSHIP BETWEEN THE ELECTRICAL RESISTANCE OF THE SOIL AND THE SPECIFIC ELECTRICAL CONDUCTIVITY OF THE PLANT-TISSUE FLUIDS

The correlations between the resistance of the soil mass as determined by the soil bridge and the specific electrical conductivity of the leaf tissue-fluids are shown in Table XII for Egyptian, for Upland and for the hybrid generation grown in 1921. The magnitudes of these coefficients are shown graphically by the position of the circles connected by solid lines on the scale of ordinates in the two parts of Figure 1.

TABLE XII.—*Correlation coefficients measuring the relationship between the electrical resistance of the soil and the specific electrical conductivity of the leaf-tissue fluids in Egyptian and Upland cotton and in their F_1 hydrid, as grown at Sacaton, Ariz., in 1921*

	N	First foot		Second foot		Third foot		Fourth foot		All layers		Average correlations for all layers
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	
Pima Egyptian from self-fertilized seed, rows 1, 5:												
First series	39	−0.3360±0.0958	3.51	−0.4430±0.0867	5.11	−0.2636±0.1004	2.63	−0.0523±0.1077	0.49	−0.3360±0.0958	3.51	−0.2737
Second series	39	+.0223± .1079	.21	−.2024± .1035	1.96	+.1951± .1038	1.88	+.0198± .1079	.18	+.0038± .1079	.04	+.0087
Difference		+.3583± .1443	2.48	+.2406± .1350	1.78	+.4587± .1444	3.18	+.0721± .1523	.47	+.3398± .1443	2.35	+.2827
Pima Egyptian, from bulk seed, rows 3, 7:												
First series	38	−.1798± .1058	1.70	−.1844± .1056	1.75	−.0115± .1094	.11	−.0155± .1093	.14	−.1295± .1075	1.21	−.0978
Second series	38	+.1765± .1060	1.67	+.1009± .1083	.93	+.2879± .1003	2.87	+1588± .1066	1.49	+.2294± .1036	2.22	+.1810
Difference		+.3563± .1498	2.38	+.2853± .1513	1.89	+.2994± .1484	2.02	+.1743± .1527	1.14	+.3589± .1493	2.40	+.2788
Pima Egyptian, from bulk seed, rows 2, 4, 6:												
First series	59	−.1011± .0614	1.65	−.1051± .0614	1.71	−.0488± .0619	.79	+.0289± .0620	.47	−.0739± .0617	1.20	−.0565
Second series	59	−.1561± .0605	2.58	−.2254± .0589	3.83	+.0608± .0618	.99	+.2402± .0585	4.11	−.0372± .0620	.60	−.0201
Difference		−.0550± .0862	.64	−.1203± .0851	1.41	+.1096± .0875	1.25	+.2113± .0852	2.48	+.0367± .0875	.42	+.0364
Meade Upland, from self-fertilized seed, rows 1, 5:												
First series	39	−.4536± .0857	5.29	−.4794± .0831	5.77	−.5129± .0795	6.45	−.3506± .0947	3.70	−.5473± .0756	7.24	−.4491
Second series	39	+.2898± .0989	2.93	+.0698± .1074	.65	+.0982± .1069	.92	+.0002± .1079	.00	+.1468± .1056	1.39	+.1145
Difference		+.7434± .1309	5.68	+.5492± .1358	4.04	+.6111± .1332	4.59	+.3508± .1436	2.44	+.6941± .1299	5.34	+.5636
Meade Upland, from bulk seed, rows 3, 7:												
First series	38	−.1382± .1073	1.29	+.0063± .1094	.08	−.1126± .1080	1.04	−.0590± .1090	.54	−.1040± .1082	.96	−.0784
Second series	38	−.1231± .1077	1.14	−.0497± .1091	.46	−.0175± .1093	.16	−.0958± .1084	.88	−.0914± .1085	.84	−.0715
Difference		+.0151± .1520	.10	−.0580± .1545	.38	+.0951± .1537	.62	−.0368± .1537	.24	+.0126± .1532	.08	+.0039
Acala Upland, from bulk seed, rows, 2, 4, 6:												
First series	59	−.0147± .0620	.24	−.1016± .0614	1.66	+.0495± .0619	.80	+.0769± .0617	1.25	+.0013± .0620	.02	+.0025
Second series	59	−.0096± .0620	.16	−.1216± .0611	1.99	+.0065± .0620	.11	+.0744± .0617	1.21	−.0167± .0620	.27	−.0125
Difference		+.0051± .0877	.06	−.0200± .0866	.23	−.0430± .0876	.49	−.0025± .0875	.03	−.0180± .0877	.21	−.0150
F_1 Hybrid rows, 1, 5:												
First series	39	−.4607± .0850	5.42	−.7380± .0491	15.03	−.4973± .0812	6.12	−.2334± .1021	2.29	−.5860± .0709	8.27	−.4826
Second series	39	+.0842± .1072	.79	−.1245± .1063	1.17	+.0992± .1069	.93	−.0629± .1075	.59	−.0027± .1079	.03	−.0010
Difference		+.5449± .1368	3.98	+.6135± .1171	5.24	+5965± .1342	4.44	+.1705± .1483	1.15	+.5833± .1291	4.52	+.4816
F_1 Hybrid rows, 3, 7:												
First series	38	−.3974± .0921	4.32	−.2097± .1046	2.00	−.2768± .1010	2.74	−.1676± .1063	1.58	−.3441± .0964	3.57	−.2629
Second series	38	+.1284± .1076	1.19	+.1602± .1066	1.50	+.0922± .1084	.85	−.0536± .1091	.49	+.1122± .1080	1.04	+.0818
Difference		+.5258± .1416	3.71	+.3699± .1493	2.48	+.3690± .1482	2.49	+.1140± .1523	.75	+.4563± .1448	3.15	+.3447

The coefficients for the first series are negative for the Egyptian plants practically throughout. Since soil resistance is expressed in ohms while the tissue fluid conductivity is expressed in ohms, a negative sign indicates a positive correlation between the actual concentrations of the electrolytes in the two solutions. For the Egyptian series the correlations range from sensibly zero to −0.44. For the Upland series the results are with but four exceptions negative in sign, and range from sensibly zero to −0.51. For the hybrid series the coefficients are negative throughout, and range from −0.17 to −0.74.

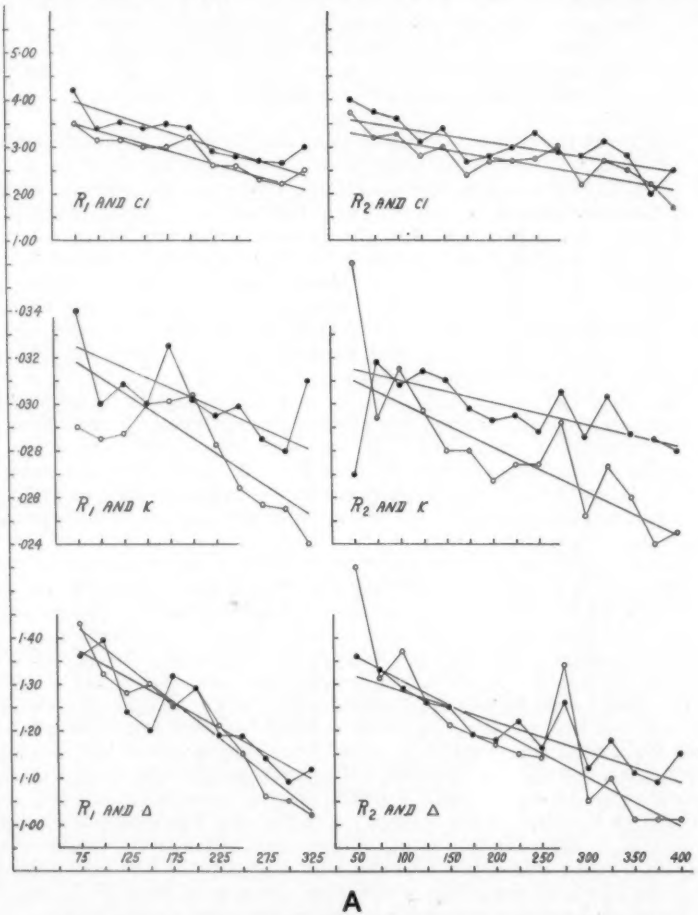
While the number of determinations on the plants is small, several of the coefficients are so large in comparison with their probable errors that there can be no reasonable doubt concerning the statistical trustworthiness of the results. The validity of this conclusion is supported by the fact that the average value of the correlation for the upper 4 feet has in seven of the eight series of determinations a negative sign, and often a substantial value. Furthermore, the correlation between the average resistance of the upper 4 feet and the electrical conductivity of the plant-tissue fluids, is in every case but one negative in sign, and in four of the cases clearly significant in comparison with its probable error.

The foregoing is the result which one might expect if differences in the salt content of the plants were determined by differences in the quantities of salts available for absorption from the soil. A remarkable fact is that these relationships do not hold in the second series. Here 24 out of 40 correlations are positive, which suggests that higher salt content in the plant sap is associated with lower salt content in the soil.

The correlations are generally much lower than those in the first series; only 5 of the 32 coefficients for individual layers of soil are more than 2.5 times as large as their probable errors. The average value for Pima from selfed seed is +0.009 in the second series, as compared with −0.274 in the first. For Pima from bulk seed the average is +0.181 in the second series, as compared with −0.098 in the first. For Meade from selfed seed the average for the second series is +0.115, as compared with −0.449 for the first. For Meade from bulk seed the average is −0.072 for the second series, as compared with −0.075 for the first. The averages for the hybrid in the second series is −0.001 and +0.082, as compared with −0.482 and −0.263 in the first. The values for Acala and Pima plants in rows 2, 4, and 6 are sensibly zero. These results leave no doubt that there is a real relationship between soil conductivity and plant-tissue fluids in the first series but a practical absence of such relationship in the second series of determinations on the plant-tissue fluids.

Turning back to Table X for the correlations between soil resistance and osmotic concentration, a similar although not as great a difference between the constants for the first and the second series of determinations may be noted. In 35 of the 40 cases in which correlation was determined, the constant for the first series is numerically more negative than that for the second series. Many of these differences may be considered individually significant in comparison with their probable errors. The statistical trustworthiness of the result for the experiment as a whole is unquestionable.

This result is all the more remarkable because of the fact that the soil constants were determined after the second series of tissue-fluid determinations had been run. One might, therefore, have reasonably expected a closer correlation between soil resistance and tissue-



A

FIG. 6.—A. Regression straight lines and empirical means showing change in freezing-point depression Δ, in specific electrical conductivity κ, and in chloride content *Cl*, associated with variation in the electrical resistance (in ohms) of the soil of the first foot R_1, and of the second foot R_2, in Meade Upland cotton, as grown at Sacaton, Ariz., in 1922. Solid dots represent the first series of determinations and circles represent the second series. (For third foot and fourth foot see fig. 6, B.)

fluid concentration in the second than in the first series of determinations. This point will be considered later.

The correlation coefficients showing the relationship between the resistance of the soil mass and the specific electrical conductivity of the plant-tissue fluids of the three varieties of cotton grown in 1922 are set forth, with their probable errors and their ratios to their

probable errors, in Table XIII. The magnitudes of the coefficients for the first series of determinations are shown by the position of the circles connected by solid lines on the scale of ordinates of Figure 2, and those for the second series are shown in Figure 3.

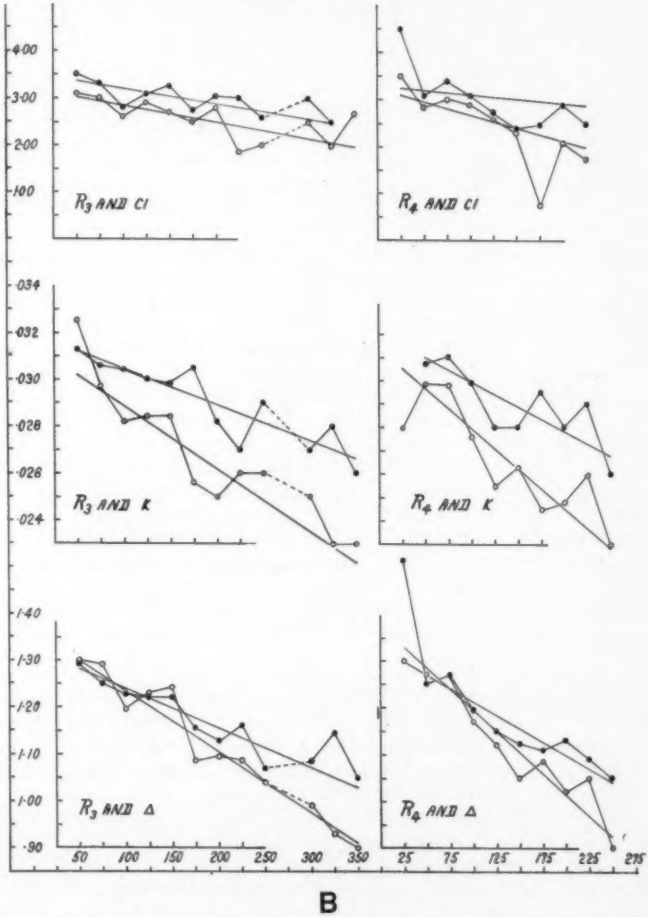


FIG. 6.—B. Regression straight lines and empirical means showing change in freezing-point depression Δ, in specific electrical conductivity κ, and in chloride content *Cl*, associated with variation in the electrical resistance (in ohms) of the soil of the third foot R_3, and of the fourth foot R_4, in Meade Upland cotton, as grown at Sacaton, Ariz., in 1922. Solid dots represent the first series of determinations and circles represent the second series. (For first foot and second foot see fig. 6, A.)

Without exception, the 30 coefficients are negative in sign. While they differ among themselves, some of the variations apparently being systematic and others chaotic, any one of the constants may reasonably be considered significant in comparison with its probable error. The coefficients as a whole may be taken to be of the order $r = 0.50$.

TABLE XIII.—*Correlation coefficients measuring the relationship between the electrical resistance of the soil and the specific electrical conductivity of the leaf-tissue fluids in Pima Egyptian and in Meade and Acala Upland cotton as grown at Sacaton, Ariz., in 1922*

	N	First foot		Second foot		Third foot	
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r
Pima Egyptian:							
First series	70	−0.5261±.0583	9.02	−0.3976±.0678	5.86	−0.4288±.0657	6.52
Second series	70	−.6410±.0474	13.50	−.5479±.0564	9.71	−.5131±.0593	8.64
Difference		−.1149±.0751	1.53	−.1503±.0882	1.70	−.0843±.0885	0.95
Meade Upland:							
First series	70	−.4055±.0673	6.02	−.3907±.0683	5.72	−.4702±.0627	7.49
Second series	70	−.4910±.0612	8.02	−.6220±.0494	12.59	−.6517±.0463	14.05
Difference		−.0855±.0910	0.94	−.2313±.0843	2.74	−.1815±.0779	2.33
Acala Upland:							
First series	71	−.4031±.0670	6.01	−.3296±.0713	4.62	−.2424±.0753	3.22
Second series	70	−.4750±.0624	7.61	−.4747±.0624	7.60	−.5290±.0580	9.11
Difference		−.0719±.0916	0.78	−.1451±.0947	1.53	−.2866±.0950	3.02

	Fourth foot		All layers		Average correlations for all layers
	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	
Pima Egyptian:					
First series	−0.4280±.0658	6.50	−0.4916±.0611	8.04	−0.4451
Second series	−.4656±.0631	7.37	−.6020±.0514	11.71	−.5419
Difference	−.0376±.0912	0.41	−.1104±.0798	1.38	−.0968
Meade Upland:					
First series	−.4580±.0637	7.19	−.4640±.0632	7.34	−.4311
Second series	−.6188±.0497	12.44	−.6525±.0462	14.10	−.5959
Difference	−.1608±.0808	1.99	−.1885±.0783	2.41	−.1648
Acala Upland:					
First series	−.3026±.0727	4.16	−.3341±.0711	4.70	−.3194
Second series	−.6088±.0507	12.00	−.5771±.0537	10.74	−.5219
Difference	−.3062±.0886	3.46	−.2430±.0891	2.72	−.2025

The most conspicuous difference is that between the constants for the two sets of determinations. These are negative in sign throughout, thus indicating that the numerical magnitude of the coefficients is higher in the second series than in the first. Many of the differences may reasonably be considered significant.

It may be noted also that the coefficients showing the relationship between the average resistance of all four layers of soil and the plant conductivities are not uniformly higher than those for the individual layers as they were found to be in the cases of freezing-point depression.

The regression-equation straight lines shown in Table XI are represented with the empirical averages for the electrical conductivity of the individual layers of soil by the uppermost figure in the two parts of Figure 4 for Pima, in the two parts of Figure 5 for Acala, and in the two parts of Figure 6 for Meade. The empirical means for the first series are represented by circles, and those for the second series by solid dots.

While there are individual irregularities, the excellent fit of the series of lines as a whole serves to emphasize two main points: (*a*) the

intimacy of the relationship between the concentration of the soil solution and the concentration of electrolytes in the plant-tissue fluids, and (*b*) the possibility of greatly extended use of the field culture method in the investigation of physiological problems which have heretofore been considered amenable only to laboratory experimentation. Determinations of both chloride and sulphate content of the soil are now being made for the purpose of extending these measures of interrelationship between the properties of the soil and the characteristics of the plant.

A comparison of the magnitude of these coefficients with those for osmotic concentration and chloride content will be given later.

Before leaving the problem of the relationship between the electrical resistance of the soil and the electrical conductivity of the plant-tissue fluids, the inconsistency between the results for the first and second series of determinations made in 1921 must be considered. As indicated above that the differences are anomalous in themselves, and they are not found in the experiment of 1922.

The most probable explanation of the result (which is not due to any detectable errors of laboratory measurement or calculation) is that of abnormalities of environmental conditions.

The irrigation of the experimental plot of 1921 was delayed somewhat beyond the time when it would have been most beneficial to the plants, in order to permit the completion of the first series of determinations in as short a period and under as uniform conditions as possible. Immediately after the heavy irrigation which was given the plot at the close of the first series of determinations there was a rainfall of more than 2 inches, which kept the soil saturated for an abnormally long period and probably influenced to a considerable degree the constants of the second series of determinations. The abnormalities of the second series are to some extent evident in both the freezing-point depressions and in the electrical conductivities. The whole problem requires special investigation.

Comparison of the Correlations Between the Resistance of the Soil Solution and the Conductivity of the Plant-Tissue Fluids, and Between the Resistance of the Soil Solution and the Freezing-Point Depression of the Plant-Tissue Fluids

The relative values of the correlations between the resistance of the soil solution and the conductivity and osmotic concentration of the plant fluids are so important physiologically that it is desirable to compare these correlations directly.[15] This is done in Table XIV for the cultures grown in 1921, and in Table XV for the cultures grown in 1922. The relative magnitudes of the coefficients may also be seen for both the first and second series of determinations in Figure 1 for the cultures of 1921, and in Figure 3 for the first series of determinations and in Figure 4 for the second series of determinations made in 1922.

[15] These tables give only the differences between the coefficients of correlations which are compared. The original coefficients are given in Tables IX for freezing-point depression and in Table XII for conductivity for the experiment of 1921. The original constants are given in Table X for freezing-point depression and in Table XIII for specific electrical conductivity for the experiment of 1922.

TABLE XIV.—*Comparison between (a) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression of the leaf-tissue fluids, $r_{R\Delta}$, and (b) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the specific electrical conductivity of the leaf-tissue fluids, r_{RK}, in Pima Egyptian and Meade and Acala Upland cotton and in their F_1 hybrid as grown at Sacaton, Ariz., in 1921. Comparison is made in terms of the difference $r_{R\Delta} - r_{RK}$*

	First foot	Second foot	Third foot
First series:			
Rows 1, 5—			
Pima Egyptian	−0.1764±0.1246	−0.1351±0.1126	−0.2574±0.1275
Meade Upland	−.0884±.1147	−.1466±.1059	−.0542±.1081
Hybrid	+.0050±.1206	+.1133±.0821	−.1033±.1066
Rows 3, 7—			
Pima Egyptian	−.3858±.1293	−.3071±.1343	−.3688±.1439
Meade Upland	−.2547±.1417	−.3619±.1454	−.1133±.1498
Hybrid	+.0599±.1337	−.1897±.1392	−.0729±.1393
Rows 2, 4, 6—			
Pima Egyptian	−.2334±.0825	−.2185±.0828	−.1250±.0863
Acala Upland	−.2465±.0848	−.2068±.0832	−.1098±.0875
Second series:			
Rows 1, 5—			
Pima Egyptian	−.2076±.1500	−.1032±.1425	−.3777±.1471
Meade Upland	−.3352±.1462	−.2789±.1489	−.0674±.1518
Hybrid	+.1508±.1480	+.1231±.1515	+.0844±.1494
Rows 3, 7—			
Pima Egyptian	−.5058±.1440	−.4160±.1464	−.3896±.1475
Meade Upland	−.1083±.1494	−.1518±.1513	−.2150±.1505
Hybrid	−.2691±.1519	−.3132±.1509	−.2456±.1522
Rows 2, 4, 6—			
Pima Egyptian	−.1791±.0818	−.1656±.0789	−.1498±.0872
Acala Upland	−.1022±.0872	−.1126±.0847	−.0428±.0877

	Fourth foot	All layers	Average
First series:			
Rows 1, 5—			
Pima Egyptian	−0.2109±0.1473	−0.2344±0.1203	−0.1950
Meade Upland	+.0484±.1364	−.0720±.1007	−.0602
Hybrid	−.1543±.1372	−.0386±.0967	−.0346
Rows 3, 7—			
Pima Egyptian	−.2546±.1491	−.4192±.1319	−.3291
Meade Upland	−.0582±.1534	−.2513±.1444	−.1970
Hybrid	+.0764±.1519	−.0307±.1346	−.0316
Rows 2, 4, 6—			
Pima Egyptian	−.0924±.0875	−.1918±.0845	−.1674
Acala Upland	−.0151±.0873	−.1890±.0862	−.1450
Second series:			
Rows 1, 5—			
Pima Egyptian	−.2316±.1492	−.2736±.1472	−.2300
Meade Upland	−.1622±.1506	−.2670±.1499	−.2109
Hybrid	−.0023±.1520	+.1128±.1517	+.0890
Rows 3, 7—			
Pima Egyptian	−.3261±.1505	−.5207±.1441	−.4094
Meade Upland	−.2200±.1465	−.2089±.1472	−.1738
Hybrid	−.0953±.1527	−.2968±.1510	−.2308
Rows 2, 4, 6—			
Pima Egyptian	−.1705±.0850	−.2082±.0851	−.1663
Acala Upland	+.0101±.0872	−.0805±.0873	−.0620

TABLE XV.—*Comparison between (a) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression of the leaf-tissue fluids, $r_{R\Delta}$, and (b) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the specific electrical conductivity of the leaf-tissue fluids, r_{Rk}, in Pima Egyptian and in Meade and Acala Upland cotton, as grown at Sacaton, Ariz., in 1922. Comparison is made in terms of the difference $r_{R\Delta} - r_{Rk}$*

	First foot	Second foot	Third foot
First series:			
Pima Egyptian	-0.1660 ± 0.0717 2.32	-0.3200 ± 0.0781 4.10	-0.2569 ± 0.0782 3.29
Meade Upland	$-.1297 \pm .0883$ 1.47	$-.1731 \pm .0874$ 1.98	$-.0650 \pm .0847$ 0.77
Acala Upland	$-.1703 \pm .0859$ 1.98	$-.2863 \pm .0869$ 3.29	$-.2889 \pm .0947$ 3.05
Second series:			
Pima Egyptian	$-.0139 \pm .0663$ 0.21	$-.1686 \pm .0689$ 2.45	$-.1632 \pm .0738$ 2.21
Meade Upland	$-.0588 \pm .0834$ 0.71	$-.0258 \pm .0683$ 0.38	$+.0351 \pm .0684$ 0.51
Acala Upland	$-.0763 \pm .0842$ 0.91	$-.1763 \pm .0779$ 2.26	$-.0683 \pm .0780$ 0.88

	Fourth foot	All layers	Average
First series:			
Pima Egyptian	-0.1387 ± 0.0853 1.63	-0.2832 ± 0.0689 4.11	-0.2204
Meade Upland	$-.0732 \pm .0857$ 0.85	$-.1148 \pm .0826$ 1.39	$-.1103$
Acala Upland	$-.2713 \pm .0903$ 3.00	$-.3051 \pm .0854$ 3.57	$-.2542$
Second series:			
Pima Egyptian	$-.1076 \pm .0834$ 1.29	$-.1461 \pm .0626$ 2.33	$-.1133$
Meade Upland	$+.0197 \pm .0719$ 0.27	$+.0040 \pm .0659$ 0.06	$-.0074$
Acala Upland	$-.0215 \pm .0704$ 0.31	$-.1029 \pm .0692$ 1.49	$-.0856$

Here differences are so taken that a negative sign indicates that $r_{Rk} < r_{R\Delta}$. The probable errors of the differences in these coefficients have been determined on the assumption that it is given by the square root of the sum of the squares of the probable errors of the two coefficients involved. Thus it neglects the correlation which probably exists between these two correlation coefficients, but as this corrective term would tend to lower rather than to raise the probable error of the difference, the conclusion that some of the differences may be considered individually significant can hardly be questioned.

These differences are negative in sign for 35 of the 40 comparisons of individual correlation coefficients in the first and second series of determinations made in 1921. The difference between the average correlation for the four layers is negative in all eight comparisons of the first series, and in seven of the comparisons for the second series, of 1921.

Because of the small number of determinations [16] available as a basis for constants it has not seemed worth while to calculate the generally low ratios of the differences to their probable errors for the constants of 1921.

[16] The number of determinations is small only when considered from the statistical standpoint. It is very large when considered from the standpoint of the usual chemical series.

In the absence of such criteria, conclusions may safely be based on the general consistency of the results for the large number of individual comparisons made.

In the comparisons based on the larger series of determinations made in 1922, ratios of the differences to their probable errors, as well as the probable errors, are given in Table XV.

With three exceptions the differences are negative in sign in the 30 individual comparisons based on the more extensive experiments of 1922. They are negative for all 6 comparisons of averages. While the ratios of these differences to their probable errors are in general small, this may be due in part to the fact that the probable errors of the differences are themselves too large, as well as to the fact that the number of measurements is not large statistically. The general consistency of the series of differences shows clearly that the correlations for the concentration of ionized salts in the soil and the concentration of all solutes in the plant-tissue fluids are larger than those for the concentration of ionized (conducting) solutes in the soil and ionized (conducting) solutes in the plant saps.

This result seems so remarkable that some other measure of the relative concentration of dissociable solutes should be obtained. This will be attempted in the following section.

The Relationship Between the Electrical Resistance of the Soil and the Chloride Content of the Leaf-Tissue Fluids

Determinations of the chloride content of the leaf-tissue fluids, carried out by the method of Lawrence and Harris (*23*), are available for the experiment of 1922 only.

The correlations between the chloride content of the associated plants of Pima Egyptian and Meade and Acala Upland cotton have already been considered (*12*) in relation to the problem of the differentiation of these two types with respect to their capacity for the absorption of anions (*13*), and in relation to the soil heterogeneity.

Turning now to the correlations between the electrical resistance of the soil mass and the chloride content of the leaf-tissue fluids, the numerical results are set forth in Table XVI and are presented to the eye by the position on the scale of ordinates of the circles connected by broken lines in Figure 2, A, for the first series and Figure 3 for the second series.

TABLE XVI.—*Correlation coefficients measuring the relationship between the electrical resistance of the soil and the chloride content of the leaf-tissue fluids in Pima Egyptian and in Meade and Acala Upland cotton, as grown at Sacaton, Ariz., in 1922*

	N	First foot		Second foot		Third foot	
		$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r
Pima Egyptian:							
First series	69	−0.7182±0.0393	18.27	−0.6987±0.0415	16.82	−0.6454±0.0473	13.63
Second series	68	−.6093±.0514	11.85	−.6576±.0464	14.17	−.6346±.0488	12.99
Difference		+.1089±.0647	1.68	+.0411±.0623	0.66	+.0108±.0680	0.16
Meade Upland:							
First series	69	−.4686±.0633	7.40	−.3892±.0689	5.65	−.2792±.0748	3.73
Second series	69	−.4730±.0630	7.50	−.4531±.0645	7.02	−.3741±.0698	5.36
Difference		−.0044±.0893	0.05	−.0639±.0944	0.68	−.0949±.1023	0.93
Acala Upland:							
First series	70	−.4595±.0635	7.23	−.5658±.0548	10.32	−.3986±.0678	5.88
Second series	70	−.4615±.0634	7.28	−.3906±.0683	5.72	−.3885±.0684	5.68
Difference		−.0020±.0897	0.02	+.1752±.0876	2.00	+.0101±.0963	0.10

	Fourth foot		All layers		Average correlations for all layers
	$r \pm E_r$	r/E_r	$r \pm E_r$	r/E_r	
Pima Egyptian:					
First series	−0.5293±0.0584	9.06	−0.7513±0.0353	21.25	−0.6479
Second series	−.5490±.0571	9.61	−.7202±.0393	18.30	−.6126
Difference	−.0197±.0817	0.24	+.0311±.0528	0.59	+.0353
Meade Upland:					
First series	−.2943±.0741	3.97	−.3786±.0695	5.44	−.3578
Second series	−.3973±.0683	5.81	−.4552±.0643	7.07	−.4244
Difference	−.1030±.1008	1.02	−.0766±.0947	0.81	−.0666
Acala Upland:					
First series	−.3451±.0710	4.86	−.4856±.0616	7.88	−.4423
Second series	−.4361±.0652	6.68	−.4342±.0654	6.64	−.4192
Difference	−.0910±.0963	0.94	+.0514±.0898	0.57	+.0231

These coefficients, like those for freezing-point depression, Δ, and specific electrical conductivity, κ, are negative in sign throughout. Since soil conductivity is expressed in terms of resistance, a negative coefficient indicates a higher chloride content associated with lower soil resistance, i. e., with more concentrated soil solution. Inasmuch as chlorides are abundant in the Sacaton soil the result is quite what might be expected on a priori grounds, had one been willing to admit that this type of investigation is capable of yielding results of such a high degree of consistency. The fact that the coefficients are from about 4 to about 24 times as large as their individual probable errors leaves no doubt as to the statistical trustworthiness of each and every coefficient. The coefficients are generally of a medium to moderately high order of magnitude. For Pima Egyptian the coefficients are above -0.609 numerically for the upper three feet of soil in both series of plants; they are slightly but perhaps not significantly lower for the fourth foot of soil; they are consistently lower in the comparable series of determinations based on Upland plants where they vary from -0.279 to -0.473. This point will be dealt with below.

The regression equations showing the change in mean chloride content associated with variation in soil resistance appear in the final section of Table XI. The last term of this equation shows that the chloride content of the leaf-tissue fluids increases from about 2 to about 10 mg. of Cl per liter for each variation of 1 ohm in the resistance of the soil solution.

The regression equations are represented with their empirical means in Figure 4 for Pima Egyptian cotton, in Figure 5 for Acala Upland, and in Figure 6 for Meade Upland cotton. While the empirical means are distributed with considerable irregularities about the theoretical straight lines, there can be no question that the correlation coefficient is a wholly legitimate measure of the interrelationship between soil resistance and chloride content of tissue fluids.

Turning back to the problem of the differences between the correlation coefficients for the Egyptian and Upland types noted above, and inspecting the upper figures of Figure 2 for the first series of determinations and Figure 3 for the second series made in 1922, it is to be noted that in every instance the Pima cotton has a numerically larger correlation coefficient than either of the associated Upland varieties. In other words, the chloride content of the leaf-tissue fluids of the Egyptian variety is more intimately related to the resistance of the soil solution than is that of either of the two Upland varieties (Acala and Meade).

In an earlier paper (*14*) it has been shown that the Egyptian type has a greater capacity for the accumulation of chlorides with the march of the season than has the Upland type of cotton. The present result shows that the chloride content of the Egyptian plants is more intimately related to the resistance of the soil solution (presumably due largely to chlorides) than is that of the Upland plants.

The results as a whole indicate that the Egyptian type has not merely a greater total chloride concentration than the Upland types, but that it accumulates chlorides more rapidly with the advance of the season (*14*), and that with respect to this character the individual plants are more intimately adjusted to variations in the chloride content of their substratum than are individuals of the Upland type.

These three differentiating peculiarities are obviously interrelated. They are being investigated further by the determination of the correlation between both the chloride and the sulphate contents of the soil and the concentration of these anions in the plant-tissue fluids.

Comparison of the Correlations Between the Resistance of the Soil Solution and the Chloride Content of the Tissue Fluids, and Between the Resistance of the Soil Solution and the Freezing-Point Depression of the Plant-Tissue Fluids

The differences between the correlations for soil resistance and freezing-point depression on the one hand, and the correlations between soil resistance and chloride content on the other, $r_{R\Delta} - r_{RCl}$, have been deduced from Tables X and XVI and are given, with their probable errors and with their ratios to their probable errors, in Table XVII.

TABLE XVII.—*Comparison between (a) the correlation coefficients measuring the relationship between (a) the electrical resistance of the soil and the chloride content of the leaf-tissue fluids, r_{RCl}, and (b) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the freezing-point depression of the leaf-tissue fluids, $r_{R\Delta}$ in Pima Egyptian and Meade and Acala Upland cotton, as grown at Sacaton, Ariz., in 1922.*

[Comparison in terms of the difference $r_{R\Delta}-r_{RCl}$]

	First foot	Second foot	Third foot
First series:			
Pima Egyptian	+0.0261±0.0573 0.46	−0.0189±0.0568 0.33	−0.0403±0.0635 0.63
Meade Upland	−.0666± .0852 0.78	−.1746± .0879 1.99	−.2560± .0941 2.72
Acala Upland	−.1139± .0832 1.37	−.0501± .0739 0.68	−.1327± .0888 1.49
Second series:			
Pima Egyptian	−.0456± .0692 0.66	−.0589± .0609 0.97	−.0417± .0657 0.63
Meade Upland	−.0768± .0847 0.91	−.1947± .0799 2.44	−.2425± .0860 2.82
Acala Upland	−.0898± .0847 1.06	−.2004± .0827 3.15	−.2088± .0860 2.43

	Fourth foot	All layers	Average
First series:			
Pima Egyptian	−0.0374±0.0797 0.47	−0.0235±0.0476 0.49	−0.0176
Meade Upland	−.2369± .0937 2.53	−.2002± .0875 2.29	−.1836
Acala Upland	−.2288± .0890 2.57	−.1536± .0777 1.98	−.1313
Second series:			
Pima Egyptian	−.0242± .0789 0.31	−.0279± .0531 0.53	−.0426
Meade Upland	−.2018± .0858 2.35	−.1933± .0796 2.43	−.1789
Acala Upland	−.1942± .0815 2.38	−.2458± .0786 3.13	−.1883

With one exception, the 30 differences between the individual constants are negative in sign. The six differences between average values of the coefficients for resistance and freezing-point depression and resistance and chloride content are all negative in sign. While, in general, the individual differences are not large enough in comparison to their probable errors [17] to be considered individually significant, the remarkable consistency of the 36 differences leaves no question concerning the soundness of the conclusion that the correlation for the electrical resistance of the soil and the concentration of all solutes in the plant-tissue fluids is higher than that for the electrical resistance of the soil and the concentration of a single (dissociated or nondissociated) anion.

COMPARISON OF THE CORRELATIONS BETWEEN THE RESISTANCE OF THE SOIL SOLUTION AND THE CHLORIDE CONTENT OF THE PLANT-TISSUE FLUIDS, AND BETWEEN THE RESISTANCE OF THE SOIL SOLUTION AND THE ELECTRICAL CONDUCTIVITY OF THE PLANT-TISSUE FLUIDS

In Table XVIII, the differences between the correlations for the electrical resistance of the soil and the chloride content of the plant-tissue fluids on the one hand, and for the specific electrical conduc-

[17] The probable errors of the differences have been calculated without regard to the possible correlation between the two correlations. This corrective term might so decrease the probable errors of the differences as to render a larger number of them statistically significant.

tivity of the plant-tissue fluids and the electrical resistance of the soil on the other, have been determined from the correlations in Tables XIII and XVI, and are given with their probable errors and their ratios to their probable errors.

TABLE XVIII.—*Comparison between (a) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the chloride content of the leaf-tissue fluids, r_{RCl}, and (b) the correlation coefficients measuring the relationship between the electrical resistance of the soil and the specific electrical conductivity of the leaf-tissue fluids, r_{RK}, in Pima Egyptian and in Meade and Acala Upland cotton, as grown at Sacaton, Ariz., in 1922*

[Comparisons in terms of the difference $r_{RK}-r_{Cl}$]

	First foot	Second foot	Third foot
First series:			
Pima Egyptian	+0.1921±0.0703 2.73	+0.3011±0.0795 3.79	+0.2166±0.0810 2.67
Meade Upland	+.0631±.0924 0.68	−.0015±.0970 0.02	−.1910±.0976 1.96
Acala Upland	+.0564±.0923 0.61	+.2362±.0899 2.63	+.1562±.1013 1.54
Second series:			
Pima Egyptian	−.0317±.0699 0.45	+.1097±.0730 1.50	+.1215±.0768 1.58
Meade Upland	−.0180±.0878 0.21	−.1689±.0812 2.08	−.2776±.0838 3.31
Acala Upland	−.0135±.0890 0.15	−.0841±.0925 0.91	−.1405±.0897 1.57

	Fourth foot	All layers	Average
First series:			
Pima Egyptain	+0.1013±0.0880 1.15	+0.2597±0.0706 3.68	+0.02028
Meade Upland	−.1637±.0977 1.68	−.0854±.0939 0.91	−.0733
Acala Upland	+.0425±.1016 0.42	+.1515±.0941 1.61	+.1229
Second series:			
Pima Egyptian	+.0834±.0851 0.98	+.1182±.0647 1.83	+.0707
Meade Upland	−.2215±.0845 2.62	−.1973±.0792 2.49	−.1715
Acala Upland	−.1727±.0826 2.09	−.1429±.0846 1.69	−.1027

Since both the coefficients under comparison are negative in sign, and the differences are taken $r_{RK}-r_{RCl}$, negative differences indicate larger (negative) correlations for soil resistance and conductivity of plant-tissue fluids than for soil resistance and chloride content of plant-tissue fluids.

The differences are in part positive and in part negative in sign. Closer examination of the values indicates that with one exception the differences for the Pima Egyptian cotton show that the correlation for soil resistance and chloride content is higher than that for soil resistance and specific electrical conductivity. A number of these differences may be statistically significant. In the case of the Upland series the differences for Acala are in agreement with those for Egyptian cotton in the first series of determinations in indicating closer relationship between soil resistance and chloride content than between soil resistance and specific electrical conductivity, but in the second series of determinations on Acala cotton the reverse is true. In 9 of the 10 comparisons based on Meade cotton the differences

suggest a higher correlation for electrical conductivity than for chloride content. In view of the fact that further investigations on this problem are under way it is unnecessary to go further than to suggest that the correlation in the Egyptian series is apparently more intimate for the chloride content than for the specific electrical conductivity of the leaf-tissue fluids. This result is in accord with the following facts, already fully established: (*a*) Greater chloride content of Egyptian plants, (*b*) a more rapid rate of accumulation of chlorides in the Egyptian types, and (*c*) the more intimate correlation between soil resistance and chloride content in this type.

RECAPITULATION AND DISCUSSION OF RESULTS

In the innumerable investigations heretofore directed toward the solution of the theoretically and practically important problem of the relationship between the characteristics of the plant organism and the properties of the soil solution,[18] the principal lines followed have been:

(*a*) Comparison of the yields or other characteristics of the crops grown on plots presumably identical in the original composition and physical properties of their soil, but treated with various fertilizers of more or less definitely known chemical composition.

(*b*) Measurement of growth rate, final size, and other characteristics of plants grown under the more or less highly refined experimental conditions of water or sand cultures.

While both of these methods are indispensable in agricultural research, much may be said in criticism of the results obtained from them as they have been employed in the past. Experience has shown that the results of plot trials as conventionally made are characterised by such a high degree of variability as to leave open to question a large proportion of the conclusions drawn from experiments hitherto reported. The application of the Pearsonian statistical methods to the data of experiments already conducted has forced upon workers the conclusion that the irregularities of most experimental fields are such as to invalidate comparisons based upon plot tests made without extensive replications and interpreted without proper statistical analysis of the data. Theoretically, greenhouse and laboratory experimentation with sand or water cultures involving definitely known compositions and concentrations of nutrient solutions should be the final court of appeal for decisions as to the relationships between the plant and the soil. Without doubt this must be true for investigations dealing with the problem of the precise rôle of specific ions of the nutrient solution. It is necessary to realize, however, that the elaborate control in such experiments may introduce abnormalities of conditions which may defeat the objects of the experiments. Students of water and sand cultures are now beginning to reconsider their technique.

When one deals biometrically with the various problems of field heterogeneity, the question must ultimately arise: Why should not the very differences which are found from place to place in any experimental field, and which are so great an obstacle to the interpretation of experiments involving the use of variously treated field

[18] In many instances distinction between the composition of the soil and that of the soil solution has not been made.

plots, be themselves made the means of investigating many of the physiological problems of the relationship between the properties of the soil and the characteristics of the plant?

The only essentials for the success of such investigations are (*a*) the tenability of the assumption that the observed differences in the soil properties are of a relatively permanent nature; (*b*) the practicability of accurately measuring the soil properties which are of significance in the determination of the plant characters; and (*c*) the availability of mathematically sound and practically usable statistical methods of dealing with the interrelationships between the two systems—the soil and the plant. The present investigation serves to establish these points by the results obtained for a specific agricultural crop (Egyptian and Upland cottons), as grown under irrigation in the arid Southwest. The findings as to matters of fact will now be briefly reviewed.

(1) In the alluvial soils of the Southwest, experimental areas of a quarter of an acre or less are markedly heterogeneous in respect of the salt concentration as measured in terms of the electrical resistance of the saturated soil mass. The heterogeneity coefficients (measured in terms of correlation) are not merely of a substantial and statistically significant magnitude, but the values are so related to the closeness of association of the subplots as to indicate the possibility of highly refined statistical investigations on the soil properties of an experimental field. This conclusion is based on the results for one measure only, but nonquantitative observations on these soils indicate that they are also heterogeneous in respect of other physical characters which may influence the characteristics of the plant.

(2) With the heterogeneity of the soil is associated a heterogeneity in the characteristics of the plant-tissue fluids of the crop covering it. There is not, however, an exact parallelism between the magnitudes of homologous coefficients in the two systems of correlations (the system of correlations for the soil and the system for the plants) but merely a general similarity of trend. The differences between the two systems are now under investigation.

Finding (1) substantiates the results of earlier investigations on the universality and relative permanence of field heterogeneity, while finding (2) extends this by indicating clearly that the heterogeneity demonstrated for crop yield in earlier studies is dependent upon the physical and chemical characteristics of the soil. Both of these results indicate that the relationship between the properties of the soil and the characteristics of the plant should be capable of direct measurement. This has been done in the present investigation by the determination of the correlation between the electrical resistance of the soil and the osmotic concentration, specific electrical conductivity and chloride content of the leaf-tissue fluids of one Egyptian and two Upland varieties as well as of the F_1 hybrid between Pima Egyptian and Meade Upland cotton.

(3) The correlations between the electrical resistance of the soil and the osmotic concentration, as measured in terms of freezing-point depression of the leaf-tissue fluids, are, in general, substantial and statistically significant. In the larger experiments, the coefficients for the upper 3 feet of soil are higher than -0.60 in both series of determinations on Pima Egyptian cotton, and higher than -0.50

for Meade and Acala Upland cotton. The correlations for the average resistance of the upper 4 feet of soil are of the order −0.75 for Pima Egyptian, and −0.60 to −0.70 for the Upland varieties. Since soil salinity is expressed in terms of the resistance of the soil mass in ohms, whereas the concentration of the plant-tissue fluids is expressed in terms of freezing-point depression, a negative correlation indicates higher concentrations for all solutes (both nonelectrolytes and dissociated and nondissociated electrolytes) of the plant-tissue fluids associated with higher concentrations of the soil solution. For all soil layers the correlation for Egyptian cotton seems to be slightly higher than that for the Upland varieties.

(4) The coefficients measuring the relationship between the electrical resistance of the soil and the electrical conductivity of the plant-tissue fluids show considerable irregularities of magnitude which presumably are due to uncontrollable abnormalities of conditions, but with a generally negative trend in the experiment of 1921. Since measures on the soil are expressed in terms of resistance, whereas those for the plant are expressed in terms of specific electrical conductivity, the negative correlation indicates that higher concentrations of dissociated (conducting) electrolytes in the soil are accompanied by higher concentrations of dissociated (conducting) electrolytes in the plant-tissue fluids. The coefficients for the larger experiment of 1922 are negative in sign and statistically significant throughout. The values range from about −0.24 to about −0.65.

(5) The correlation between the freezing-point depression of the plant-tissue fluids and the resistance of the soil is higher than that between the electrical conductivity of the plant-tissue fluids and the resistance of the soil. In short, there is a more intimate relationship between the concentration of all solutes in the plant-tissue fluids and the conducting solutes of the soil mass than between the electrolytes (conducting solutes) of the plant-tissue fluids and the conducting solutes of the soil. This result opens up a number of physiological problems which are receiving further attention.

(6) There is a substantial and statistically significant correlation between the resistance of the soil mass and the chloride content of the plant leaf-tissue fluids for Egyptian plants. The coefficients are over −0.60 for the upper 3 feet of soil. For the Upland plants the coefficients vary from −0.28 to −0.40 for the same layers. Thus the chloride content of the leaf-tissue fluids of the Egyptian variety is more intimately related to the resistance of the soil solution than is that of either of the two Upland varieties (Acala and Meade) considered. Taken in connection with earlier investigation, this result indicates that the Egyptian type has not merely a greater chloride concentration than the Upland type and that it accumulates chlorides more rapidly with the advance of the season, but that the individual plants are more intimately adjusted to the variations in the chloride content of their substratum than are individuals of the Upland type. These three differentiating peculiarities are obviously interrelated.

(7) The correlation between soil resistance and the concentration of chlorides in the plant-tissue fluids is, in practically every instance, lower than that between soil resistance and the concentration of all solutes in the plant-tissue fluids as measured by the freezing-point depression.

(8) The correlation between soil resistance and the chloride content of the plant-tissue fluids is higher than that between the soil resistance and the concentration of dissociated (conducting) solutes in the plant-tissue fluids in the case of the Egyptian cotton; but the two sets of coefficients are of so nearly the same magnitude in the Upland varieties that further work will be necessary to determine whether there is a difference between them.

SUMMARY

In summarizing it may be noted that (1) and (2) above substantiate and extend findings from earlier investigations on the physical basis of the heterogeneity of the experimental field and its influence upon the crop. The results stated under (3) to (8) have a twofold significance. First, they contribute to the system of physiological data which is being built up for one of the important crop plants of our arid regions. They will be fully understood only when considered in relation to other papers on these problems. Second, they indicate clearly the possibility of carrying out in the field many important physiological investigations which have been supposed to be capable of investigation only under controlled laboratory conditions.

The relationships considered here are merely illustrative. There is no reason why they should not be greatly extended, both as to the soil properties and as to the plant characters dealt with. For example, the relationship between the specific salts to which soil concentration or reaction is due on the one hand and yield and quality of product on the other should be readily investigated by these means. It is important to bear in mind that the present investigation was carried out on a small experimental plot. The variability in physical conditions which is shown by such an area can be greatly increased only by increasing the size of the tract.

Since the relationship between soil properties and plant characteristics can be measured in terms of correlation and be expressed by a regression curve, it may be possible in the future to predict more precisely than is possible now the character of the crop which would be produced by a field of given characteristics in a normal year.

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A STUDY OF SMUT RESISTANCE IN CORN SEEDLINGS GROWN IN THE GREENHOUSE[1]

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INTRODUCTION

The development of disease-resistant varieties of plants through hybridization and selection obviously is one of the most important and permanent means of controlling plant diseases. Smut caused by *Ustilago zeae* is one of the most destructive diseases of corn and one for which no practical method of control has been discovered. In recent years various investigators have found marked differences in the degree of resistance of different strains of corn to smut. The possibility of developing desirable smut-resistant varieties is encouraging.

In a breeding program for developing resistant strains or varieties, the greatest assurance lies in the wise selection of individuals or strains from a large number of plants grown under conditions where maximum infection by the fungus is certain. In the field the possible maximum infection seldom occurs except when artificial inoculation is practiced, and often not then. By inoculating the soil and plants in various ways with chlamydospores and with conidia from pure culture, fairly satisfactory results have been obtained in most years. A partial or total loss of even one year's results is a serious handicap in a program of this kind, which at best must necessarily proceed slowly. In view of this, the need for a quicker and more certain means of testing corn plants for smut infection is obvious.

The object of the investigations reported herein was to find a means of testing corn seedlings for resistance to smut. If it were found that young seedlings could be tested, large numbers could be handled in comparatively small space by removing them as soon as the records were made. In view of the fact that infection of corn by smut in the field is influenced greatly by climatic factors, it was decided to conduct this investigation in the greenhouse, where certain of these factors could be controlled, and where corn seedlings could be tested through the winter months if a successful method of inoculation were developed. Furthermore, information could be obtained on the resistance of the progeny of important strains of corn before time for planting them in the field the following season, thus hastening the separation of undesirable and desirable strains.

PREVIOUS INVESTIGATIONS

The literature dealing with the infection of corn by *Ustilago zeae* under field conditions is extensive. There are, however, very few records of studies of this kind made in the greenhouse. Most in-

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vestigators are agreed that, under field conditions, corn seedlings are less susceptible than plants 1.5 to 2 feet and more in height. Brefeld [2] and Kühn [3] both were able to obtain infection of a few seedling plants in the field, but in all cases the plants were killed by the smut. Piemeisel [4] marked a number of naturally infected corn seedlings in the field, and found that a majority of them finally were killed by the smut, while a few of them grew to maturity and produced ears.

In 1899 Arthur and Stuart [5] reported successful infection of corn seedlings by *Ustilago zeae* in the greenhouse. They germinated the seeds between moist cloths and sprayed the seedlings with conidia. The radicles were about 1 inch and the plumules about 1.5 inches long when inoculated. After spraying, the seedlings were kept between the moist cloths for 24 hours and then transplanted to pots and boxes in the greenhouse. Of 9 plants, 6 showed signs of infection in 11 days. Three of the 6 died and the other 3 developed well-defined lesions.

Piemeisel,[4] who found it difficult to produce infection of corn seedlings in the field, makes the following statement:

> Further inoculations of germinating seeds were made in the greenhouse. Forty-three seedlings were dipped into a water suspension of sporidia and then planted in pots. None of the plants developed smut, although they were allowed to grow for one and one-half months.

Apparently neither Arthur and Stuart nor Piemeisel considered the probable effect of temperature on infection, as this factor is not mentioned by them.

In the winter of 1921–22 the senior writer conducted an experiment, in the greenhouse at a temperature of 65° to 75° F. similar to that conducted by Piemeisel.[4] Silver King corn, furnished by F. D. Richey, agronomist in the Office of Cereal Investigations, was used in these experiments. On November 1, 1921, five rows each of smut-free (rows 1 to 5) and inoculated (rows 6 to 10) seed were planted on a greenhouse bench at Arlington Experiment Farm. Sixteen kernels were planted in each row. On the same date a quantity of seed was planted in sand, and on November 5 the resulting seedlings were transplanted to a greenhouse bench after being treated as follows: (1) Seedlings dusted with chlamydospores; (2) seedlings dipped in a water suspension of conidia from pure culture; (3) plumules wounded and seedlings dipped in suspension of conidia; (4) seedlings not inoculated.

On January 7, 1922, of the plants in rows 1 to 5 grown from clean seed, those in row 1 were inoculated by dropping a water suspension of chlamydospores of *Ustilago zeae* into the buds. The plants in rows 4 and 5 were inoculated in the same way with conidia in suspension. The conidia used on the plants in row 4 came from a culture obtained from Wisconsin, while those used on the plants in row 5 came from a culture of *Ustilago zeae* isolated from a smut gall from California. Carrot agar was used for culturing the smut for these experiments.

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On January 27, 1922, the plants in all the above experiments were inspected and all were free from smut. The temperature probably was too low for infection to take place.

In 1924, Sartoris [6] failed to obtain infection of corn seedlings grown in sterile test tubes and in the greenhouse by inoculating with mycelium of *Ustilago zeae* from pure culture. He regulated moisture conditions but did not mention temperature, which is one of the chief factors limiting infection. However, Sartoris is very positive in his conclusion that "the mycelium obtained in culture is unable as such to attack the host."

PRESENT INVESTIGATIONS

These present studies were conducted at the Arlington Experiment Farm of the United States Department of Agriculture at Rosslyn, Va., and at the Kansas Agricultural Experiment Station at Manhattan, Kans. Excluding the experiments in 1921–22, reported under "Previous Investigations," plantings for the first experiments were made by the senior writer at Arlington Farm on November 1, 1923. On February 12, 1924, the junior writer began experiments independently at Manhattan. The results of these experiments are here combined. Although the data from either place are limited, they give some indication of the possibilities of testing corn in the seedling stage in the greenhouse for its resistance to smut. Furthermore, they show the possibility of making such a test during the winter season, when corn can not be grown in the field.

MATERIALS AND METHODS

SEED

The use of corn highly susceptible to smut was known to be necessary in studying infection methods. It was desirable also that there be used a strain or strains sufficiently pure to remain constant in their susceptibility. For this reason inbred pure lines and hybrids from inbred strains were used. In addition to the susceptible strains, a few moderately resistant and a few highly resistant strains were included, in order to determine their reaction to smut infection under greenhouse conditions in case the methods employed were successful. One or more years' records of smut infection in the field were available for the strains selected for these studies. The seed was furnished by C. H. Kyle, agronomist, and A. M. Brunson, associate agronomist, of the Office of Cereal Investigations, United States Department of Agriculture, with whom the writers are cooperating in field studies at Arlington Experiment Farm and at Manhattan, Kans., in breeding corn for smut resistance. The strains used in the experiments at Arlington Farm were inbred strains of Garrick, Cuban Yellow, and Boone County White. Inbred strains of Australian and Commercial White and a number of F_1 and F_2 hybrids between different inbred strains of Commercial White and between strains of Commercial White and Pride of Saline and Kansas Sunflower were used at Manhattan.

[6] SARTORIS, G. B. STUDIES IN THE LIFE HISTORY AND PHYSIOLOGY OF CERTAIN SMUTS. Amer. Jour. Bot. 11: 617-647, illus. 1924.

INOCULATION

A number of methods of inoculation were employed. In some experiments chlamydospores were used, while conidia from pure cultures were used in others. The smut fungus was cultured on various types of media, but it finally was found that the most convenient way of obtaining an abundance of conidia which could be handled easily was to grow the organism in carrot decoction. Where a solid medium (carrot agar) was used, the conidia were hard to remove for preparing a water suspension, while all that was necessary where a carrot decoction was used was to pour the culture into the desired quantity of water or use it undiluted. Reasonably fresh cultures were found to cause the higher percentages of infection. After cultures become a few weeks old they generally contain fewer conidia and more mycelium and are less virulent. In the experiments on Arlington Farm in the winter of 1923–24, the conidial suspensions were made by mixing cultures of smut from a number of widely separated localities in the United States, in order to increase the chances of obtaining a virulent inoculum. This was done because differences in rate of growth and virulence seemed to exist in similar cultures of the organism from different localities.

Where chlamydospores were used they were spread over the soil, after planting the seed, either in barnyard manure or alone, or dropped into the buds of the plants in water suspension. The inoculations with conidia were made by spraying or dropping a conidial suspension into the apical buds and by hypodermic injection through the leaves to the apical bud. This was best accomplished by inserting the syringe needle into the tissues below the bases of the uppermost leaves and injecting the suspension until the water was seen to rise in the leaves and even drip from their axils. The latter method was found to be the most successful. After inoculation, the plants either were placed in moist chambers or left on the greenhouse benches. In the greenhouse at Arlington Farm, 12 glass chambers, each large enough to hold six 9-inch pots, were available for these studies. These chambers were constructed on a side bench in the greenhouse and were provided with screened ventilators near the top.

The type of moist chamber used in the experiments at Manhattan was one employed there in leaf-rust studies. It consisted of a galvanized-iron cylinder without top or bottom. The cylinder was placed on end on moist sand or soil, and the top of the cylinder was covered with glass. The plants were placed inside where the humidity was maintained at about 100 per cent.

EXPERIMENTS AT ARLINGTON EXPERIMENT FARM, 1923-24

IN GLASS CHAMBERS

Plantings for the first greenhouse experiments at Arlington Farm were made on November 1, 1923. The 6 pots in each of 6 of the glass chambers, numbered 1 to 6, were planted, 2 kernels per pot, to an inbred susceptible strain (240–460–405) of Boone County White. Seed from the same supply planted in the field in 1923, where 96.3 per cent of the plants were infected after artificial inoculation, was planted in these pots.

Two days after planting the seed in the glassed chambers, fresh horse manure mixed with chlamydospores of smut collected on Arlington Farm the previous season was spread over the pots in chamber 1. Chlamydospores alone were dusted over the pots in chamber 2. On November 14, spores were dusted over the plants in chambers 1 and 2. On November 21, no signs of infection could be seen on any of the plants. At that time the plants in chambers 1, 2, 3, 4, and 5 were inoculated by dropping a water suspension of conidia from pure culture into the apical buds. On January 3, 1924, no signs of infection could be seen. The plants were then inoculated by injecting a conidial suspension into the buds with a hypodermic syringe. The conidia were obtained from tube cultures on carrot agar. Cultures of the smut fungus from seven States—California, Florida, Kansas, Pennsylvania, Texas, Virginia, and Wisconsin—were mixed for these inoculations, for the purpose already stated.

On January 18, large smut galls were found on 2 plants in chamber 1, and on 1 plant in chamber 2, and a small gall on one plant in chamber 5. The temperature in the greenhouse had been maintained at 65° to 75° F. A steam leak under the bench, which increased the moisture and temperature in chambers 1 and 2, seemed to favor the development of the smut.

The temperature was then, January 18, raised so as to range from 70° to 95° F. (generally around 80° to 85° F.), and the house was sprayed lightly three to six times daily to keep the humidity high. The plants in all six chambers were inoculated by injecting a suspension of conidia from mixed cultures. Thirteen days later smut galls were present on a high percentage of the plants. Etiolated areas or blotches, thought at the time to be infections, but not definitely known to be such, were noted on plants without galls as well as on those which did have them. Plants with only these spots were not listed as infected. The fact that these blotches occurred only on parts of the plant near the point of inoculation indicated that they were infections. Similar blotches were found later, through a microscopic study, to contain a mycelium similar to that found in smut galls. The results of the inoculations of January 18 are given in Table I. However, these plants, being about 2½ months old, had passed the true seedling stage. Percentages of plants producing galls are given in Table I and in other tables in this paper where only plants with galls were considered as infected. Percentages of infection are not given in the other tables, as they would mean very little in view of the reaction of the different strains of corn after infection takes place.

TABLE I.—*Infection of strain 240–460–405 of Boone County White corn, grown at an average temperature of 80° to 85° C. with high humidity in glassed chambers in the greenhouse at Arlington Experiment Farm in 1924, after inoculation by injecting a suspension of conidia from mixed culture into the buds*

Chamber No.	Number of plants		Percentage with galls	Chamber No.	Number of plants		Percentage with galls
	Inoculated	Galls formed			Inoculated	Galls formed	
1	8	4	50.0	4	9	7	77.8
2	7	2	28.6	5	5	3	60.0
3	4	4	100.0	6	7	5	71.4

The data in Table I show that under these greenhouse conditions, where the moisture and temperature are kept high, infection of corn plants of this age, about 2½ months, can be obtained successfully by injecting the conidia between the leaves in the apical buds of the plants.

ON GREENHOUSE BENCH

On November 1, 1923, at the same time that the seed was planted for the experiments in the chambers, seed of a number of inbred strains was planted on a wide bench in the center of the greenhouse for inoculation studies. Later plantings were made on January 3 and February 2, 1924, in which seed of these same strains and a few others was used. A list of these inbred strains, with their varietal names and their smut records in the field for the four years preceding these experiments, are given in Table II.

TABLE II.—*A list of inbred strains of corn used in the smut-infection studies, in the Arlington Experiment Farm greenhouse, in 1923–24, with their percentages of smut infection in the field for the preceding four years*

Variety	Strain No.	Percentages of field infection in—			
		1920	1921	1922	1923
Garrick	207–99–256	38.0	15.0	40.0	49.50
Do	207–99–51	18.0	1.0	55.0	35.48
Do	207–54–30	20.0	0.0	0.0	10.64
Do	207–86–49	22.0	0.0	0.0	15.76
Do	207–54–30	20.0	0.0	0.0	19.50
Cuban Yellow	218–69–305	35.0	23.0	65.0	97.98
Do	218–69–189	21.0	7.0	33.3	91.58
Do	218–79–319	33.0	1.0	3.0	27.37
Boone County White	240–577–416	43.0	0.0	0.0	15.05
Do	240–577–414	37.0	1.0	2.7	12.37
Do	240–460–405	39.0	12.0	30.0	96.30

The data in Table II show that some of the inbred strains of corn used in these experiments in 1923–24 were fairly resistant, while others were moderately to highly susceptible under field conditions. The high percentages of infection in 1923 are due in part to artificial inoculation. None of the strains used was as resistant as one or two strains used in the following season in similar experiments, the results of which are given later in this paper.

On November 3, 1923, two days after eight of the above strains of corn were planted, the soil was inoculated by spreading spore-charged manure over the surface. On November 14, dry spores were dusted over the young seedlings and the soil. On November 21, a suspension of conidia from pure culture was dropped into the buds of the plants. On January 3, 1924, one plant of strain 218–69–305 had a few small smut galls on the leaves. Several of the plants of each strain were inoculated at this time (January 3) by injecting a suspension of conidia from mixed cultures through the leaves to the apical buds with a syringe, and another group of plants of each strain was inocu-

lated by dropping the same type of inoculum into the buds. On January 18, one slight smut infection was found in one plant inoculated by the injection method. At this time one group of the plants of each strain was again inoculated by the injection method. The temperature was then raised, as previously stated, and the house subsequently sprayed three to six times daily to keep the humidity high. On February 8, the rest of the plants were inoculated by the same method. The data in Table III show the results of these inoculations. The infections could be seen as etiolated blotches six days after inoculation.

TABLE III.—*Infection of inbred strains of corn planted November 1, 1923, in the Arlington Experiment Farm greenhouse, produced by injecting a suspension of conidia from mixed cultures from various sources*

Strain No.	Inoculated Jan. 18, 1924			Inoculated Feb. 8, 1924		
	Number of plants—		Percentage of infection	Number of plants—		Percentage of infection
	Inoculated	Infected		Inoculated	Infected	
207-99-256	10	3	30.0	7	7	100
207-99-51	8	0	0	None.		
218-69-305	12	4	33.3	6	4	66.7
218-69-189	11	1	9.1	2	0	0
218-79-319	12	5	41.7	5	3	60.0
240-577-416	11	0	0	12	10	83.3
240-577-414	12	0	0	11	9	81.8
240-460-405	10	3	30.0	5	3	60.0

In the plantings made on January 3, 1924, the strains listed in Table III and three additional strains were used. Twelve plants of each strain were inoculated, on January 18, by dropping a conidial suspension into the buds. Eleven days later there were no signs of infection. After this a number of plants in each strain were inoculated by the injection method on three different dates. Mixed cultures, as previously described, were used in each case. The results of these inoculations are given in Table IV. The signs of infection were noted in most cases about five to six days after inoculation. The white blotches, or early indications of infection, were noted on plants of all strains, regardless of their degree of susceptibility in the field. A distinction in degree of infection is noted in the table in the results of inoculations on February 8 and March 10. In most cases the strains which were most susceptible in the field were more severely injured by smut in the greenhouse, even though the actual percentages of infection were no higher. The term "other symptoms" is used in Table IV and some of the following tables to include such symptoms as blotching, stunting, and the presence of minute pimples, where there was no noticeable gall formation.

TABLE IV.—*Infection of corn plants in the Arlington Experiment Farm greenhouse by Ustilago zeae, produced by injecting a conidial suspension into the buds of the plants. Seed planted January 3, 1924*

Strain No.	Number of plants inoculated on different dates							
	Jan. 29		Feb. 8			Mar. 10		
	Inoculated	Infected *	Inoculated	Infected		Inoculated	Infected	
				Galls	Other symptoms		Galls	Other symptoms
207–99–256	9	0	11	1	0	12	0	4
207–99–51	11	1	12	0	0	13	0	3
218–69–305	12	5	12	2	6	16	4	7
218–69–189	12	4	12	1	5	15	4	5
218–79–319	12	7	10	2	4	15	2	6
240–577–416	11	2	11	1	10	15	0	8
240–577–414	12	3	12	3	9	15	0	10
240–460–405	6	1	12	11	0	12	2	4
207–54–30	12	0	12	1	2	15	0	5
207–86–49	8	2	7	0	2	10	2	2
207–54–30	10	0	12	1	5	7	1	6

* No records of degree of infection were made on plants inoculated Jan. 29.

The data in Table IV show that corn plants are susceptible in the seedling stage as well as in later stages of development, under these greenhouse conditions. They show that strains which have a higher degree of susceptibility in the field are also more susceptible in the greenhouse. Strains 218–69–305, 218–69–189, and 240–460–405 were highly susceptible in the field and they showed a high degree of susceptibility in these experiments.

Seed of 4 strains of corn from the list given in Table II was planted February 2, on a bench in the greenhouse. The plants were emerging five days later. On March 10, about half of the plants of each strain were inoculated by the injection method, using condia from mixed cultures. The records of these inoculations, made 12 days later, are given in Table V. In no case did smut develop on uninoculated plants. For this reason they are not considered in the tables.

TABLE V.—*Infection of corn seedlings by Ustilago zeae in the Arlington Experiment Farm greenhouse, recorded on March 22, produced by injecting a conidial suspension into the buds of the plants on March 10, 1924*

Strain No.	Number of plants		
	Inoculated	Infected	
		Galls	Other symptoms
240–577–414	29	7	22
240–460–405	12	9	3
240–577–416	25	10	14
207–99–256	25	14	7

The data in Table V show that high percentages of infection were obtained in plants of all four of the strains of corn inoculated. The percentages of plants producing galls, however, were somewhat lower in strains 240–577–414 and 240–517–416, which had previously shown a higher degree of resistance in the field. Observations made on these plants on April 4 showed that the gall development was much less pronounced on these strains than on the other two. The invasion of the tissues by the smut fungus evidently was checked through some internal reaction on the part of the host plant. A microscopic study of infected tissues revealed the presence of the fungus in these resistant strains as well as in the more susceptible plants.

EXPERIMENTS IN KANSAS IN 1924 AND 1925

In the similar experiments at Manhattan, in 1924, the first plantings were made on February 12, and additional plantings were made every 5 and 6 days until four plantings had been made, in order to have seedlings of different ages for the inoculations. Strain 1054×5 (an F_1 hybrid of two smut-susceptible lines) and strain 1066×8 (an inbred strain of Australia dent corn) were grown for these studies. Ten pots of each strain were planted, with one seed per pot on each date of planting.

The smut cultures used for the inoculation of these plants were pure cultures on carrot agar, isolated about a year previously from smut galls from Kansas. Transfers of the organism to the tubed carrot agar were made 3 days before using.

Two methods of inoculation were employed. In one method a suspension of conidia in carrot decoction was sprayed with an atomizer, principally on the older tissues of plants. In the other method, three drops per plant of the conidial suspension was dropped by means of a finely drawn medicine dropper into the apical buds in direct contact with the tender growing tissues.

Seedlings of four different ages, namely, 12, 18, 23, and 28 days, were inoculated by each of these two methods. Those of each age, subjected to each method of inoculation, were divided into two equal groups. One group was placed in a cool greenhouse at 55° to 65° F., while the other was placed in a warmer house with a temperature of 75° to 85° F. Under each of these temperatures, two seedlings, one inoculated by each of the two methods, were placed in moist chambers, while comparable ones were left on benches in the greenhouses. The plants on the benches were not watered for 48 hours, while those in the chambers were kept in a saturated atmosphere for 48 hours and then removed and placed near those left on the benches. Uninoculated plants were placed with the inoculated ones for controls. The results of this experiment are given in Table VI. None of the controls developed smut under any condition, so they are not included in the table.

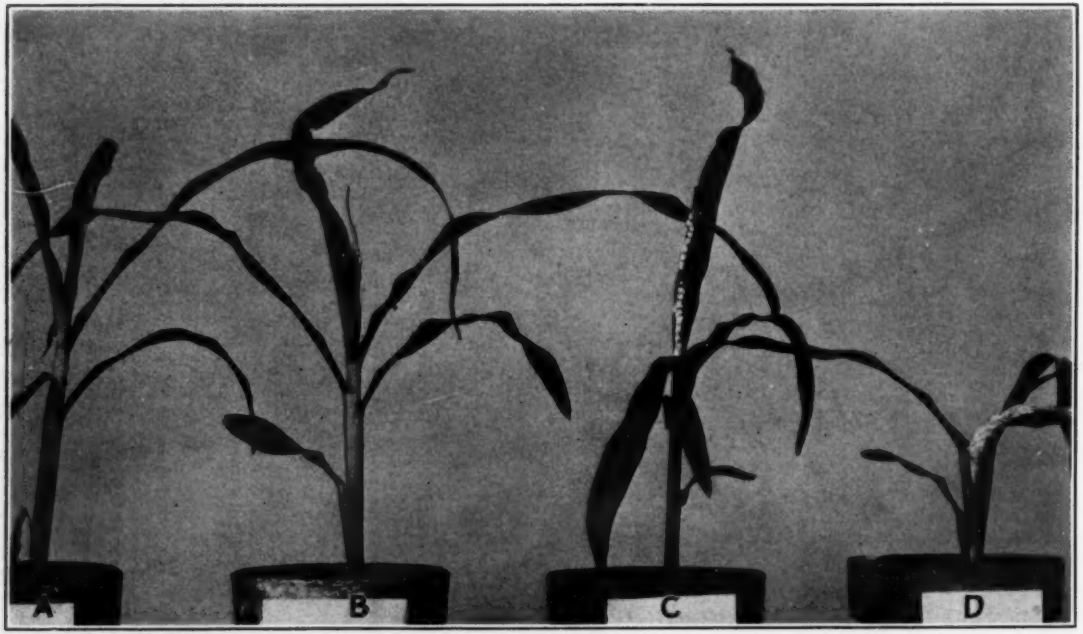
TABLE VI.—*Infection of corn seedlings produced by artificial inoculation with Ustilago zeae under different conditions of temperature and moisture in the greenhouse at Manhattan, Kans. in 1924. (One plant of each age in each experiment)*

Strain No.	Age of plants in days	Plant infected or uninfected when inoculated by—								Total number of plants	
		Spray method				Dropping in bud					
		In chamber		On bench		In chamber		On bench			
		75 to 85° F.	55 to 65° F.	75 to 85° F.	55 to 65° F.	75 to 85° F.	55 to 65° F.	75 to 85° F.	55 to 65° F.	Infected	Inoculated
1054×5	12	Yes	Yes	No	Yes	Yes	Yes	Yes	No	6	8
1054×5	18	Yes	No	No. pl.	No	Yes	No	No	Yes	3	7
1054×5	23	No	No	No	No	Yes	No	No	Yes	2	8
1054×5	28	Yes	No	No	No	Yes	Yes	No	No	3	8
1066×8	12	No	No	No	No	Yes	Yes	No	Yes	3	8
1066×8	18	No	No	No	No	Yes	Yes	Yes	No	3	8
1066×8	23	No	No	No	No	Yes	Yes	Yes	Yes	4	8
1066×8	28	No	No	No	No	Yes	No	No	No	1	8
Total		3	1	0	1	8	5	3	44	25	63

The data in Table VI show that dropping the conidia into the buds was much more effective than spraying them on the seedlings. They show also that more infection took place in plants incubated in moist chambers. The higher temperature also was more effective in producing infection. Although distinct symptoms of smut infection were evident on many of the plants, few of them developed smut galls. The results were not as satisfactory as desired, due to the lack of heavy infection under the conditions of the experiment most suited to cause infection and to the lack of the development of distinct smut galls. It is thought that perhaps the smut culture used for the inoculations might have been too old to be sufficiently viable to infect readily, even though it produced a large number of conidia in culture.

Another culture of *Ustilago zeae* was obtained, by the plate method of isolation, from a smut gall from Idaho. This fungus grew much more rapidly in culture than did the fungus used in the previous experiment. Transfers made from carrot agar cultures 2 weeks old to carrot decoction showed by microscopic examination, after 3 days' growth in the decoction, that the organism from Idaho had produced about four times as many conidia as the fungus from Kansas. This difference in production of conidia may have been due to the inherent nature of the organisms, to a difference in age of the cultures, or to some other cause.

A second group of plants was inoculated, using the Idaho smut culture. The same strains of corn of four different ages were used. Plants 11, 15, 19, and 24 days old were inoculated. Since the best results in the previous experiment were obtained by dropping the inoculum into the bud and incubating the plants in the warmer greenhouse, the spray method and the cooler house were not used. The inoculations were made on March 28. The apical bud of each plant was filled with inoculum, the water which had collected in the buds having been first shaken out. The apical buds of check plants were filled with sterile carrot decoction. Some of the inoculated



Types of infection obtained in corn seedlings of strain 1054 × 5 by *Ustilago zeae*, produced by filling the apical buds with a conidial suspension, and growing the plants in a greenhouse at 75° to 85° F. (at Manhattan, Kans.). Photographed 12 days after inoculation

A.—Leaf blotched and frayed
B.—Leaf blotched and frayed, and a few small smut galls on central leaf
C.—Definite smut lesions on central leaf
D.—Heavy smut infection, which caused the seedling to become deformed

and check plants of each strain of corn was then placed in moist chambers for 48 hours, while the rest of each group was left on the bench. The temperature of the greenhouse averaged about 80° to 85° F.

The first signs of infection were noted 5 days after inoculation. The symptoms were typical of those noted in previous experiments. The infections advanced rapidly, and at the end of 10 days several plants showed definite smut galls (pl. 1, C, D). Many of these increased in size very rapidly, eventually killing the plants. The inoculated plants all showed evidence of infection, while only one control plant showed an abnormal condition which might have been confused with smut infection. The results of these inoculations are shown in Table VII.

TABLE VII.—*Infection of corn seedlings by Ustilago zeae, produced by filling the buds with carrot decoction charged with conidia, and growing them in the greenhouse at Manhattan, Kans., in 1924*

Strain No.	Age of plants in days	Number of plants grown—					
		In moist chamber			On bench		
			Infected			Infected	
		Inoculated	Galls	Other symptoms	Inoculated	Galls	Other symptoms
1054×5	11	2	1	1	2	2	0
1054×5	15	3	3	0	3	3	0
1054×5	19	3	0	3	3	0	2
1054×5	24	5	0	5	None.		
1066×8	11	2	2	0	2	1	0
1066×8	15	2	1	1	2	0	2
1066×8	19	2	1	1	2	0	2
1066×8	24	4	0	3	None.		

The data in Table VII show that only slight advantage was gained by use of the moist chambers. This was perhaps due to the large quantity of inoculum used, which did not dry out in either case until infection had taken place. Considering the degree of infection, young plants were more susceptible to smut than older plants. Strain 1054×5 seemed to be a little more susceptible than strain 1066×8.

Further experiments were conducted at Manhattan in 1925 for the purpose of confirming some of the previous results. Several inbred strains and hybrid selections of corn were used for these experiments. Transfers from cultures of the Idaho smut used the previous year were used. The same methods of culturing the fungus on carrot agar decoction were employed. Although it was previously thought that old cultures were less viable, this inoculum proved effective. The plants were inoculated by two methods. In one the inoculum was forced down into the buds of the plants by a slender glass tube operated on the principle of a medicine dropper. In the other method the inoculum was injected hypodermically by inserting the needle into the side of the plant about an inch above the ground. The seedlings were inoculated in the 2-leaf to 4-leaf stage. The plants were grown in 3-inch and 4-inch pots. The results of these inoculations are given in Table VIII.

TABLE VIII.—*Infection of corn seedlings produced by artificial inoculation with Ustilago zeae in the greenhouse at Manhattan, Kans., in 1924–25*

Strain of corn	Date of inoculation	Number of plants grown								
		In moist chamber						On bench		
		Inoculum forced into bud			Inoculum injected hypodermically			Inoculum injected hypodermically		
			Infected			Infected			Infected	
		Inoculated	Galls	Other symptoms	Inoculated	Galls	Other symptoms	Inoculated	Galls	Other symptoms
B	1924 Dec. 31				15	0	15	15	0	15
B	1925 Jan. 3				20	0	20	20	0	20
183	Feb. 2	10	0	1	10	0	4			
183	Feb. 12	15	11	3	15	7	2			
6 × 23	do				16	5	1			
50 × 4	do				9	7	2			
183	Feb. 20	20	18	1	20	19	0			
6 × 23	do	20	17	2	20	19	1			
50 × 4	do	20	18	2	20	2	0			

The figures in Table VIII show again that corn seedlings can be infected by smut under the given conditions. In no case did any of the control plants show signs of infection.

In another experiment at Manhattan, several F_2 selections of crosses between different inbred strains of Commercial White corn and between inbred strains of Commercial White and Pride of Saline and Kansas Sunflower were planted on January 19, 1925, on a bench, in rows about 8 inches apart, in the warm greenhouse at 80° to 85° F. The seedlings were inoculated hypodermically when in the 2-leaf and 3-leaf stages, 16 and 22 days after the seed was planted, and left undisturbed under the moisture conditions normally existing in the warm greenhouse. The data in Table IX show the results of these inoculations. None of the control plants showed signs of infection.

TABLE IX.—*Infection of F_2 corn seedlings from seed planted January 19 in the greenhouse at Manhattan, Kans., and inoculated February 5, 1925, by a hypodermic injection of a conidial suspension of Ustilago zeae*

INOCULATED IN 2-LEAF STAGE (16 DAYS OLD)

Strain No.	Number of plants					
	Inoculated	Infected, shown by—				Total
		Large galls	Leaf pimples	Stunting	Blotching	
271	20	0	0	3	3	6
270	34	1	0	5	3	9
269	27	0	1	4	12	17
268	27	0	0	13	6	19
267	31	0	0	8	3	11
265	28	0	1	4	7	12
264	32	0	0	15	15	30
263	31	0	1	8	5	14
262	36	0	1	17	4	22
242	28	0	1	14	6	21

TABLE IX.—*Infection of F_2 corn seedlings from seed planted January 19 in the greenhouse of Manhattan, Kans., and inoculated February 5, 1925 by hypodermic injection of a conidial suspension of Ustilago zeae*—Continued

INOCULATED IN 3-LEAF STAGE (22 DAYS OLD)

Strain No.	Number of plants					
	Inoculated	Infected, shown by—				Total
		Large galls	Leaf pimples	Stunting	Blotching	
241	29	0	3	8	0	11
240	24	10	7	3	0	20
239	25	2	2	13	1	18
238	28	6	3	3	0	12
237	21	1	5	3	4	13
236	24	1	0	6	0	7
234	21	0	5	6	1	12
233	22	3	0	9	0	12
232	26	2	3	5	0	10
231	23	1	5	7	0	13

The data in Table IX show that more smut galls were produced in the plants inoculated in the 3-leaf stage. This might have been due to the vigor of the plants or to other strain differences. All the seed was planted on the same date, and the inoculations were made 16 and 22 days later. Some strains were apparently more susceptible to infection than others. This difference in susceptibility in the greenhouse seemed to bear little relation to the difference in susceptibility noted in parental lines of these strains in the field.

EXPERIMENTS AT ARLINGTON EXPERIMENT FARM IN 1924–1925

IN GLASS CHAMBERS

In further studies in the greenhouse at Arlington Farm, seed of two susceptible inbred strains of Garrick corn were planted November 20, 1924, in pots, in 6 of the glass chambers already mentioned. Seed of both strains were furnished by C. H. Kyle, and were of his selection No. 90. Chambers 1 to 4 contained strain 90–53, and chambers 5 and 6 contained strain 90–54. Chambers 1, 3, and 5 each had several burlap bags on the floor and one hung on the wall. These bags were kept wet by spraying the chambers several times daily in order to keep the air saturated. Chambers 2, 4, and 6 were watered just often enough to keep the soil sufficiently moist for the best growth of the plants.

On December 2, the seedlings in chambers 1 and 2 were inoculated by dropping into the apical buds the conidia from fresh carrot-decoction cultures of smut from Arlington Experiment Farm. The seedlings in chambers 3 to 6 were inoculated with similar cultures of smut from Dalhart, Tex. In each case the smut cultures were 6 days old. In both instances the inoculations were repeated in the same way with the same smut cultures 2 days later. None of the seedlings had advanced beyond the 3-leaf stage. Of the 18 seedlings in chamber 1, 3 had distinct smut galls and 1 other showed symptoms

on December 16, 12 days after inoculation. No signs of infection could be seen on the seedlings in the other chambers. These results seemed to indicate that the smut from Arlington was more viable than the smut from Texas, and that the humid conditions in the moist chamber favored infection.

On December 16, the seedlings in chambers 3, 4, 5, and 6 were inoculated by the injection method with a 20-day-old culture of smut from Texas. The data in Table X show the results of these inoculations. The records were made January 6, 1925.

TABLE X.—*Infection of corn seedlings inoculated by hypodermic injection of conidia of Ustilago zeae, December 16, 1924, and grown in moist and dry chambers in the greenhouse at Arlington Experiment Farm, Va.*

Chamber No.	Condition of chamber	Number of plants		Percentage with galls
		Inoculated	Galls formed	
3	Moist	17	9	52.94
4	Dry	16	7	43.75
5	Moist	12	8	66.67
6	Dry	4	2	50.00

The data in Table X show again that the conditions in the moist chambers were slightly more favorable for infection. However, the difference was not great, due perhaps to the fact that sufficient inoculum was injected to keep the conidia moist, even in the dry chambers, for a sufficient time to permit infection to take place. No doubt the moisture conditions surrounding the plant have little effect so long as the inoculum is kept supplied with water until infection takes place. After these experiments it was decided to continue to keep the greenhouse humid for infection studies in order to prevent too rapid drying out of the inoculum.

ON GREENHOUSE BENCH

On November 20, 1924, and February 19, 1925, plantings of 6 inbred strains of Garrick and 2 inbred strains of Cuban Yellow corn were made in the greenhouse at Arlington Experiment Farm in order to determine the practicability of testing corn for smut resistance by the methods which had been developed. Among the strains used were some which had proved highly resistant under field conditions and others which were very susceptible. They were planted on a bench, in rows 12 inches apart. The temperature was kept around 80° to 90° F., and the house was kept humid by frequent spraying. Cultures of smut from Arlington Experiment Farm and from Dalhart, Tex., were used as inoculum. Plants of different ages were inoculated. The injection method was used principally. Plants inoculated by dropping the inoculum into the buds failed to become infected with few exceptions. The results were much better where the inoculum was injected with a syringe. The results of the inoculations by injection of these plants are presented in Table XI.

TABLE XI.—*Infection of seedlings of inbred strains of Garrick and Cuban Yellow corn produced in the greenhouse at Arlington Experiment Farm, Va., by injecting conidia of Ustilago zeae, in 1925*

Variety and strain	Percentage of smut in field, 1924	Age when inoculated, days	Smut from—					
			Arlington Farm, Va.			Dalhart, Tex.		
			Number of plants inoculated	Infected		Number of plants inoculated	Infected	
				Galls	Other symptoms		Galls	Other symptoms
Garrick:								
90-51	67.1	12	10	5	3			
90-51	67.1	19	7	2	3	12	10	2
90-51	67.1	26				10	1	4
54-26	0	12	12	3	9			
54-26	0	19	8	0	4	12	1	8
54-26	0	26				12	0	0
90-53	54.9	12	12	3	9			
90-53	54.9	19	12	6	5	12	9	3
90-53	54.9	26				9	8	0
54-29	0	19	12	2	9	12	1	6
54-29	0	26				11	0	0
90-54	67.2	19	11	11	0	8	4	3
90-54	67.2	26				10	4	3
54-35	0	19	11	2	9	12	3	4
54-35	0	26				12	0	0
Cuban yellow:								
69-75	51.8	19	11	11	0	12	10	1
69-75	51.8	26				12	8	4
79-90	0	19	12	6	3	11	3	7
79-90	0	26				11	7	3

The data in Table XI show that very young seedlings of resistant strain 54–26, inoculated 12 days after planting, were about as susceptible as seedlings of susceptible strains 90–51 and 90–53 of the same age (pl. 2 A, B). These seedlings were inoculated when the plumules were just unfolding. Inoculation of seedlings of the same strains one week later when they were in the three-leaf stage showed strain 54–26 to be much more resistant than the other two strains (pl. 3, A, B). By studying the data of this table it is evident that resistance is manifested in the seedling stage in the greenhouse after the plants have reached the three-leaf stage. Careful observation of the seedlings after inoculation was more convincing than the result in the table, due to the fact that the galls on the resistant plants often failed to develop further after a few days. The slight symptoms tended to disappear. The smut, in many cases, continued to develop in plants of the susceptible strains, and killed many of them (pl. 3, A, B, C, D).

Considering the degree of infection on the strains of corn used, the smut obtained from Arlington Farm was somewhat more virulent than smut from Dalhart, Tex. This might have proved different if other strains of corn had been inoculated.

The results of these experiments indicate that there is a possibility of obtaining valuable information regarding the resistance of corn by inoculating seedlings in the greenhouse, where the temperature and humidity are kept high.

Infection of corn seedlings by *Ustilago zeae* (in Arlington Experiment Farm greenhouse), produced by injecting conidia hypodermically. High temperature (80° to 85° F.) and humidity maintained. Plants 12 days old when inoculated. Photographed eight days after inoculation

A.—Two seedlings of an inbred, resistant strain (54-26) of Garrick. Note galls at base of plants and on sheath and leaves. Plants of this strain showed more resistance when older (see pl. 3 A, B)

B.—Three seedlings of a susceptible strain (90-51) of Garrick. Note heavy leaf infection

A and B.—Plants of same two strains of Garrick shown in Plate 2; planted at the same time as those there shown, and inoculated by the same method one week later. Photographed three weeks after inoculation

C and D.—Infection of inbred strains of Garrick corn by *Ustilago zeae* (in greenhouse, Arlington Experiment Farm). Inoculated by the injection method 19 days after the seed was planted. Photographed three weeks after inoculation

A.—Two rows of resistant strain 54-26. Of the 12 plants inoculated, 9 showed signs of infection, and 1 had a slight gall

B.—Two rows of susceptible strain 90-51, showing smut galls on leaves and at base of several plants. Of the 12 plants inoculated, all were infected; 10 had galls, and 5 died as a result of the infection

C.—Two rows of susceptible strain 90-54. Of the 11 seedlings, 10 were killed by smut, and the other developed leaf galls

D.—Two rows of resistant strain 54-35. Of the 11 seedlings, all showed infection, but only 2 developed galls

DISCUSSION

In the greenhouse corn seedlings were found to be as susceptible to infection by smut (*Ustilago zeae*) as were older plants. Very young seedlings seemed to be even more susceptible. No doubt the reasons for the failure of most previous investigators to obtain infection of seedlings both in the greenhouse and the field was due to a lack of the moisture and temperature conditions which favor infection. Under field conditions the high temperatures which favor infection occur after the plants have passed the seedling stage if the corn is planted at the time best suited to the production of a normal yield. Apparently there has been a misinterpretation of the facts. No doubt, what is escape through natural environmental protection has been termed resistance on the part of the seedlings. If it were true that corn plants were resistant through the seedling stage and became susceptible after becoming 1.5 to 2 feet high, it would be an interesting phenomenon in view of the fact that only very young or meristematic tissues are easily infected. In the case of certain weak parasites which attack the older tissues of their host plants after the latter have completed growth, one would not expect the seedlings to be susceptible. *Ustilago zeae*, however, behaves in the opposite way.

Not only have corn seedlings been found to be susceptible, but the varietal and strain differences existing in the field with regard to resistance and susceptibility also exist under greenhouse conditions. Highly resistant strains are more heavily infected (pl. 2) in the very early seedling stage. In some of these experiments highly resistant strains showed high percentages of infection, but it is remarkable that the seedlings were able to overcome the attacks of the fungus. In many cases galls never were formed, while in the susceptible strains many of the seedlings were killed by smut invasion (pl. 3). A comparable condition is often found in the field where resistant plants show blotching and minute galls on the leaves, but the infection fails to develop further.

The fact that the seedlings of resistant strains become infected is nothing unusual in view of what has been learned regarding resistance of other plants to their respective parasites, namely, that resistance is internal and physiological rather than morphological. Microscopic study of sections of infected leaf tissues of resistant and susceptible seedlings showed the smut fungus to be present in both. Some internal reaction on the part of the resistant plant holds the fungus in check within the plant.

Differences in the ability of smut from different sources to infect corn seedlings was noted. A difference in appearance of these organisms on the same culture medium also was observed. Some cultures produced conidia more abundantly than others. These differences may have been due to the occurrence of strains of the smut fungus or to other causes such as the physiological effects of environmental and host factors to which they previously had been subjected.

The results of these experiments, although limited, are encouraging in view of the fact that it will be possible to test corn in the seedling stage in the greenhouse during the winter months. While it may not be possible to test large numbers of strains, it will be feasible to test the more important ones in the breeding program before the seed is

planted in the spring. At the same time, the conditions for infection no doubt can be made more severe than those existing in the field. In this way a valuable smut record of a given progeny can be made available during the winter, whereas under field conditions it could not be known until about time for self-pollination and hybridization to begin the following summer, and not then unless conditions for infection were favorable. Large numbers of plants can be tested under controlled conditions in comparatively small space. There may be a very valuable application of this method in a program of breeding corn for smut resistance.

SUMMARY

Methods have been devised at Arlington Experiment Farm, Rosslyn, Va., and at Manhattan, Kans., for infecting corn seedlings with *Ustilago zeae* for the purpose of studying varietal and strain resistance to smut in the greenhouse.

Fresh cultures of *Ustilago zeae* in carrot decoction have been found to be a more satisfactory inoculum than chlamydospores or conidia from cultures on solid media.

The most effective method of inoculation was the injection of a conidial suspension hypodermically. Fairly satisfactory results were obtained, in some instances, by dropping the inoculum into the apical buds, but poor results were obtained by spraying.

Cultures of smut from some sources were somewhat more effective than cultures of the fungus from other sources. The most effective individual cultures used were from Idaho and Virginia. The smut from different sources also developed and produced conidia at different rates when cultured.

High temperatures (80° to 95° F.) were found to favor infection.

Sufficient moisture to prevent drying of the inoculum until infection took place was found necessary. Slightly better results were obtained in a moist house or chamber, or by the use of a large quantity of suspension for inoculation.

Corn seedlings were found to be as susceptible as older plants, under the conditions of the experiments.

Plants showing resistance in the field also showed resistance in the greenhouse when inoculated during or after the three-leaf stage. Susceptible strains were equally susceptible in the greenhouse.

Plants of resistant strains were infected, especially while very young, but were able to suppress the invasion by the fungus to a certain extent, while susceptible plants often were killed.

DEVELOPMENT IN IMMATURE BARLEY KERNELS REMOVED FROM THE PLANT [1]

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INTRODUCTION

In a previous paper [2] the writers reported that 9 out of 10 barley seeds germinated when harvested five days after they were pollinated. These kernels had been harvested in the head and allowed to air-dry in paper envelopes. At the same time other kernels had been plucked from the head and placed in small glass vials and allowed to air-dry, while still others had been divested of lemma and palet and killed and fixed in acetic alcohol for sectioning. Similar samples had been taken for all other ages up to 10 days after pollination. When the kernels picked from the head on sampling were tested, the viability was zero in all samples taken on the fifth to eight days from pollination. Of the 9-day kernels, 3 out of 10 germinated. Furthermore, one of the nonviable kernels from the lot harvested in the head four days after germination was dug up and sectioned, and its embryo (pl. 1, A) showed a much later stage of development than did the seed of the same age (pl. 1, B) killed at harvest. The indications were that development had continued in the air-dried head sample after it was taken. At Aberdeen, Idaho, in the summers of 1922 and 1924, and at Arlington Experimental Farm, Rosslyn, Va., during the winter of 1924–25, more material was obtained and studied.

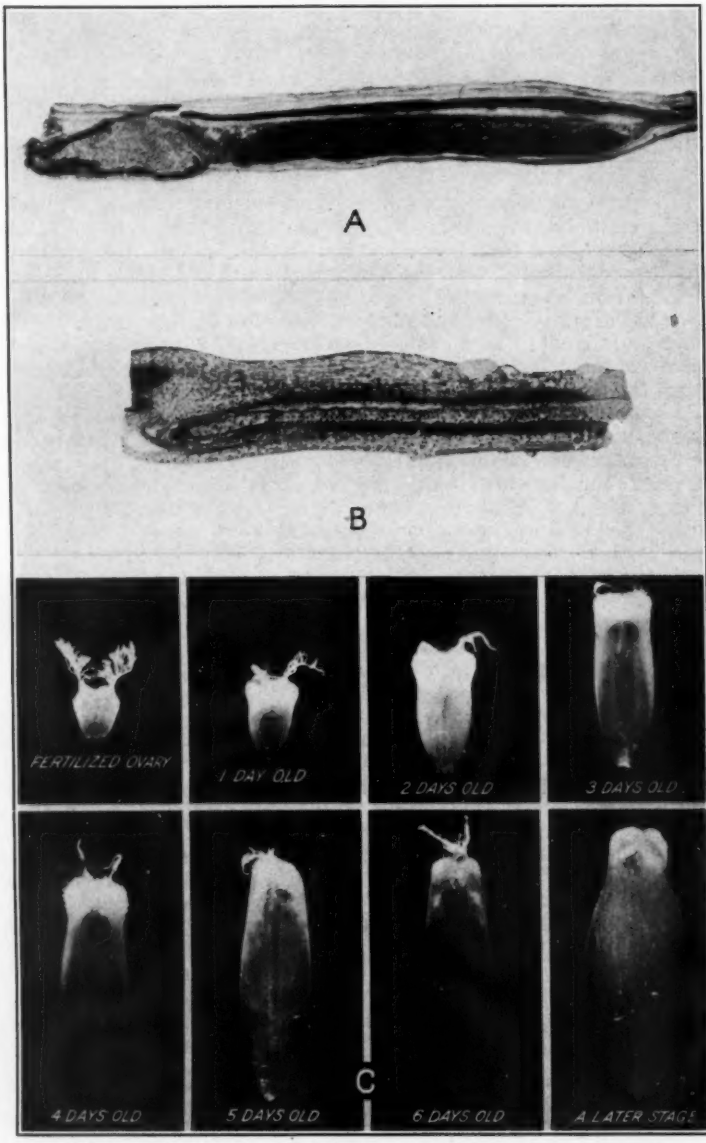
NORMAL GROWTH OF BARLEY KERNELS

The early growth of the barley kernel is very rapid (pl. 1, C). Elongation is the most noticeable feature of increased size up to 8 days after pollination, about which time the maximum length is reached. Growth continues in the lateral plane until about the fifteenth day. Under favorable conditions, the "filling" of the kernel during the latter stage of development takes place on either side of the crease, and the kernel increases in dorso-ventral diameter up to the time of near maturity, which is 26 days after pollination at Aberdeen, Idaho.

Two days after pollination the kernel is slightly less than 4 mm. long. At the end of the third day the kernel measures slightly more than 6 mm., and after four days of growth the kernel is slightly less than 8 mm. in length. The embryo by this time has become ovoid, and is about 0.12 mm. long. Up to the fifth day after pollination, there is practically no visible differentiation of tissues in the young

[1] Received for publication Sept. 30, 1925; issued March, 1926. This study was made in cooperation with the Idaho Agricultural Experiment Station.

[2] HARLAN, H. V., and POPE, M. N. THE GERMINATION OF BARLEY SEEDS HARVESTED AT DIFFERENT STAGES OF GROWTH. Jour. Heredity 13: 72-75, illus. 1922.



A.—Sagittal section of a barley kernel, four days old, cured in the head. × 23
B.—Sagittal section of a barley kernel, four days old, killed and fixed when taken from the plant. × 23
C.—Successive stages in the normal development of the barley kernel

embryo. Differentiation first appears five days after pollination, as a slight indentation of the ab-endospermal surface, with an accompanying convexity just below. At this time the kernel measures 8.8 mm. in length. At the age of seven days, the embryonic plumule and radicle are visible in the embryo.

MATERIALS AND METHODS

The variety Hannchen was chosen for this study because it is a two-rowed barley and its seed development has been carefully studied.

As the kernels in this study were sampled during the period of most rapid elongation, any growth after sampling can be clearly demonstrated by measurements of lengths in kernels of the same age. The selection of kernels of the same age is made easier by the fact that many florets in the middle of the spike are pollinated on the same day. If the florets of the four basal and the four terminal nodes are discarded, the remaining kernels are of practically the same age. An occasional exception may be found, yet Table I shows that the lengths of the kernels from the same spike measured at the time of collecting never varied by more than 0.8 mm., and this extreme variation occurred in but two cases in a total of 28. When one considers the fact that during this period of growth the normal kernel elongation for a whole day is 2.12 mm. on the third day and 1.86 mm. on the fourth day after pollination,[3] the assumption of equality in age is justified. The writers have compared only (1) hand-pollinated kernels of the same age, (2) kernels from the middle of a single head, or (3) measured kernels falling inside limits which are well within the increase in size for 24 hours.

In all cases, checks of the samples taken were divested of lemma and palet, and either dried and weighed or killed and fixed for microscopic study.

For investigating the possibility of growth, kernels were dried or fixed at intervals after sampling.

In determining the source of the dry matter in the embryo, five treatments were given as follows:

1. The culm was cut near the ground and immediately stored in a moisture-saturated atmosphere.
2. The culm was cut near the ground and allowed to air-dry in a well-ventilated laboratory under the arid conditions of southeastern Idaho.
3. Kernels from the middle of the spike were taken from heads of nearly the same age and dried in envelopes without removing the lemma and palet.
4. For this and the following treatment (5) a large number of kernels were removed from the lemma and palet and two lots were selected, one measuring 6.2 to 6.8 mm. and the other 7.2 to 7.8 mm. in length. The shorter length would mean an age slightly in excess of three days and the longer an age slightly less than four days. Kernels from each lot were laid on a dry filter paper and placed in an envelope to dry.
5. Kernels from each lot described in (4) were laid on filter paper which rested on moist cotton in a Petri dish. At a later date the treatment was slightly modified, in that the kernels were placed on a dry glass plate elevated above a layer of wet cotton in the bottom of a Petri dish.

[3] HARLAN, H. V. DAILY DEVELOPMENT OF KERNELS OF HANNCHEN BARLEY FROM FLOWERING TO MATURITY AT ABERDEEN, IDAHO. Jour. Agr. Research 19: 406, Table II. 1920.

Kernels killed and fixed for microscopic examination were embedded in paraffin. Sagittal sections 10 μ thick were made and stained with iron alum, haematoxylin, and safranin (quick method). The drawings were made with the aid of a camera lucida.

DEVELOPMENT OF THE KERNELS AFTER HARVEST

THE ENDOSPERM

In 1922, at Aberdeen 28 immature heads were gathered. Kernels 8, 9, and 10, numbered from the base on the odd side (the side of the head bearing the first spikelet), were immediately dehulled, the length measured, and the kernels killed and fixed. The remainder of the spike, to which probably a foot of culm was attached, was then wrapped in a moist towel and laid away in a root cellar at a temperature between 58° and 60° F. On the first day after sampling, kernels 6 and 7, on the even side of the spike, were dehulled, measured, and preserved as on the day before. Similarly, on the second, third, and fourth days, two kernels were measured and preserved each day. The lengths in millimeters of all the kernels fixed on each of these days appear in Table I.

The data in Table I show that the kernels of only six heads failed to increase in length in the four days following harvest. In these six heads the kernels had almost or entirely reached the limits of elongation when harvested, and measurements of the lateral and dorsoventral diameters, which were not taken, would have been necessary to show growth. Where the lengths had reached 8.5 mm. or more before the initial measurement was taken, the growth in length was much slower and the increase for the four days proportionately reduced. All of these spikes in which the kernels showed an average increase in length less than 1 mm. likewise show an average initial kernel length of 7.25 mm. or more.

TABLE I.—*Length (in millimeters) of barley kernels kept moist after harvest*

Head No.	Days after harvest											Average length		Increase in length
	0			1		2		3		4				
	Length of kernel No. (odd side)			Length of kernel No. (even side)		Length of kernel No. (even side)		Length of kernel No. (even side)		Length of kernel No. (odd side)		On fourth day	At harvest	
	8	9	10	6	7	8	9	10	11	7	11			
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
103	3.6	3.6	3.5	3.5	3.8	5.2	5.4	5.4	5.4	6.0	6.6	6.30	3.57	2.73
104	4.4	4.3	4.6	5.0	5.0	5.3	5.7	5.9	5.1	7.1	6.3	6.70	4.43	2.27
105	4.5	4.8	4.5	5.0	5.5	6.5	6.4	6.4	5.6	7.1	6.6	6.85	4.60	2.25
106	4.1	4.3	4.1	4.0	4.6	5.7	5.2	5.4	5.3	5.9	5.5	5.70	4.17	1.53
109	6.4	6.8	6.7	6.6	6.9	7.8	7.8	8.0	8.1	8.3	8.2	8.25	6.63	1.62
111	5.4	5.9	6.2	6.3	6.4	7.0	7.0	7.6	7.1	6.9	7.5	7.20	5.83	1.37
116	8.8	8.7	8.3	8.9	8.8	9.4	9.3	8.9	9.2	9.1	8.6	8.85	8.60	.25
117	8.4	8.2	8.5	8.0	8.2	8.6	8.9	9.1	9.1	8.9	8.8	8.85	8.37	.48
118	8.0	8.2	8.4	8.7	8.7	8.8	9.2	9.1	9.0	8.9	9.1	9.00	8.20	.80
121	9.8	9.4	9.4	9.4	9.5	9.7	9.6	9.4	9.8	9.9	9.5	9.70	9.53	.17
122	9.2	9.0	9.4	9.5	9.3	9.7	9.7	9.1	8.6	9.4	9.0	9.20	9.20	[a].00
125	8.6	9.2	8.6	8.0	8.2	8.6	8.8	8.8	8.8	8.1	8.7	8.40	8.80	[a]—.40
128	8.2	7.9	8.0	8.6	8.5	9.1	9.1	9.0	9.1	8.8	8.5	8.65	8.03	.62
129	8.1	7.9	7.8	7.3	7.9	8.3	7.9	9.0	8.9	9.0	8.9	8.95	7.93	1.02
130	8.6	8.5	8.3	8.3	8.7	9.1	8.5	8.6	8.7	8.7	9.1	8.90	8.47	.43
131	8.5	8.5	8.2	8.3	8.3	8.4	8.5	8.3	8.1	8.2	8.2	8.20	8.40	[a]—.20
132	8.6	9.3	8.9	8.3	8.6	8.7	9.2	9.3	[b]9.0	8.8	[b]8.7	8.75	8.93	[a]—.18
134	10.1	10.0	9.7	9.3	9.6	9.8	9.5	9.8	9.9	9.6	9.3	9.45	9.93	[a]—.48
135	7.1	7.4	7.3	6.9	7.1	7.7	7.9	8.0	7.6	8.4	7.8	8.10	7.27	.83
138	9.9	10.0	10.0	9.8	9.9	10.0	10.0	10.0	9.3	10.0	9.3	9.65	9.97	[a]—.32
139	4.3	4.1	4.0	3.9	4.2	5.4	5.0	6.1	6.2	6.1	6.6	6.35	4.13	2.22
141	3.4	3.7	3.3	3.6	3.8	4.6	4.8	5.7	5.6	5.9	5.8	5.85	3.47	2.38
143	6.5	6.3	6.4	7.2	7.1	7.9	7.8	8.2	8.1	8.1	8.2	8.15	6.40	1.75
145	5.1	5.0	5.1	5.7	5.3	6.5	7.1	7.6	7.5	6.8	7.0	6.90	5.07	1.83
150	4.6	4.6	4.5	4.5	4.5	5.8	6.0	6.2	6.4	7.0	7.0	7.00	4.57	2.43
152	5.3	5.4	5.5	5.8	5.6	6.4	6.5	7.3	7.5	8.2	7.5	7.85	5.40	2.45
158	7.2	6.8	7.4	7.3	7.4	8.8	8.4	8.5	8.3	8.9	8.9	8.90	7.13	1.77
159	5.9	6.3	6.7	5.3	6.8	7.9	7.7	8.3	8.1	7.3	.....	7.30	6.30	1.00

[a] Limit of growth probably reached at harvest.
[b] In head 132 the kernels for day 3 are 11 and 12 odd, and for day 4 are 7 odd and 10 even.

The effect of spikelet location on rate of growth is also evident in these data. In head 135, for instance, kernel 11 odd, measured on the fourth day, was close enough to the tip to be influenced in the relative amount of food material obtained by distance from the culm and by rachis structure. These differences in relative nutrition with differences in location also may be seen in the data from many other heads on the third and fourth days, as kernels 11, even and odd, were all so near the tip that they were small in size. Kernel 7, on the other hand, being better located for the delivery of nutriment from the culm, was much larger, showing what probably more nearly approximates normal growth. The extreme tip kernels also were probably a day younger than those found farther down on the spike. Consequently, the measurements of growth indicated by the difference between the averages of kernels 7 and 11 are quite conservative.

In March, 1925, a number of heads of barley bearing florets emasculated and hand-pollinated to obtain kernels of the same age were cut from the plant about a foot below the spike. When sampled, the length measurements ranged between 7 and 9 mm., and as little increase in length could be expected, growth was measured by weighing. The check kernels were removed and rapidly dried. Some of the remaining kernels were dehulled, some were left in the lemma and palet, and others were left undisturbed on the culm.

All three lots were then held in a moisture-saturated atmosphere for eight days. On the eighth day they were removed, dehulled, and partially dried. All, including the initial sample, were then held for about three weeks in a constant humidity and temperature chamber. The dry matter contents were then roughly comparable, although the kernels did contain some moisture. At the end of this period they were all carefully weighed on a chemical balance. The weights are given in Table II.

TABLE II.—*Weight (in grams) of hand-pollinated kernels gathered March 26, 1925*

	Weight when dried at time of sampling	Weight when kept for 8 days in water-saturated atmosphere		
		Peeled	Inclosed in lemma and palet	Left on culm
	Gm.	*Gm.*	*Gm.*	*Gm.*
Pollinated Mar. 9	0.0083	0.0080	0.0088	0.0178
	.0094	.0083	.0080	.0183
	.0094			.0175
				.0157
Pollinated Mar. 12	.0071	.0047	.0071	.0191
	.0050	.0079	.0068	.0194

The three kernels from the March 9 pollination, which were dried at harvest, were quite close together in weight, indicating the uniformity of the material obtained by the method used. The data also show that at harvest all the kernels from the pollination of this date weighed somewhat less than 0.01 gram. The kernels dehulled and kept moist for eight days did not increase in weight, and the same is true of the ones cured in the lemma. The kernels left on the culm, however, increased in weight markedly. The same facts appear in the weights of the kernels pollinated on March 12.

THE EMBRYO

Kernels of the 1922 material grown at Aberdeen were sectioned and studied. The embryos showed a considerable development in size and differentiation of tissues after harvest. In 1924 a larger number of kernels were harvested for study. This material, gathered three to five days after pollination, was given the five treatments described under "Material and Methods," last paragraph. Samples were killed and fixed every two days for eight days.

In Table III are given the lengths and lateral diameters of all embryos examined. The lengths were found with the aid of an eyepiece micrometer, and the lateral diameters by multiplying 10 μ by the number of sections in which the embryo appeared. Embyro sections typical for the day of harvest are shown in Figure 4, for treatments 1, 2, and 3; but all the embryos obtained in treatments 4 and 5 are shown on account of the variations occurring in them.

TABLE III.—*Measurements of barley embryos to show growth after sampling*

Treatments	Length in millimeters					Lateral diameter in millimeters				
	At time of sampling	2 days later	4 days later	6 days later	8 days later	At time of sampling	2 days later	4 days later	6 days later	8 days later
A. Kernels kept moist in head on culm:										
Head 1	0.1472	0.4045	0.9384	1.6148	1.7410	-------	0.19	0.46	-------	-------
Head 2	.1683	.4207	.9384	1.5193	1.8380	-------	.16	.47	.69	-------
Head 3	.0647	.2233	.5663	1.0517	1.5856	-------	.14	-------	.50	-------
B. Kernels dried in head on culm in laboratory:										
Head 1	.0906	.2556	.4045	.6472	1.0857	0.06	.11	.20	.41	-------
	.0858	.2411	.4045	.6181	1.0193	.05	.10	-------	.26	-------
Head 2	.0680	.2120	.4191	.7297	1.1682	.06	.09	.10	.33	-------
	.0938	.2443	.5048	.9190	1.1213	.05	.12	.22	.37	-------
Head 3	.1294	.3398	.6585	.9449	1.2119	.07	.15	.26	.28	-------
	.1440	.3252	.6294	1.0210	1.2976	.08	.16	.29	.39	-------
C. Kernels dried in lemma and palet	.1133	.3301	.7928	1.0080	1.0048	.08	.16	-------	-------	-------
	.1084	.4272	.7491	1.0064	1.0646	.06	.21	.28	-------	-------
	.1586	-------	-------	.9789	1.3462	.09	-------	-------	-------	-------
	.0777	-------	-------	-------	-------	.06	-------	-------	-------	-------
Average	.1145	.3786	.7709	.9978	1.1385	.0725	.185	.28	-------	-------
D. Kernels "peeled" and kept moist:										
Lot 1. Length 6.2 to 6.8 mm. at time of gathering	.1747	.2912	.2670	.2945	.2993	.08	.16	.19	.15	.18
	.1440	-------	-------	.3883	.5016	.10	-------	-------	.18	.21
	.1181	-------	-------	-------	-------	.07	-------	-------	-------	-------
	.2039	-------	-------	-------	-------	.10	-------	-------	-------	-------
	.1327	-------	-------	-------	-------	.09	-------	-------	-------	-------
Average	.1547	.2912	.2670	.3414	.4004	.088	.16	.19	.165	.195
Lot 2. Length 7.2 to 7.8 mm. at time of gathering	.2346	-------	.4530	.7362	.7831	.13	-------	.16	.35	.35
	.2815	-------	.3398	.5825	.5275	.11	-------	.22	.39	.21
	.2961	-------	-------	-------	-------	.11	-------	-------	-------	-------
	.1747	-------	-------	-------	-------	.09	-------	-------	-------	-------
	.1893	-------	-------	-------	-------	.10	-------	-------	-------	-------
Average	.2352	-------	.3964	.6593	.6553	.108	-------	.19	.37	.28
E. Kernels "peeled" and dried:										
Lot 1. Length 6.2 to 6.8 mm. at time of gathering	.1747	.1618	-------	-------	.1699	.08	-------	-------	-------	-------
	.1440	.2540	-------	-------	-------	.10	-------	-------	-------	-------
	.1181	-------	-------	-------	-------	.07	-------	-------	-------	-------
	.2039	-------	-------	-------	-------	.10	-------	-------	-------	-------
	.1327	-------	-------	-------	-------	.09	-------	-------	-------	-------
Average	.1547	.2079	-------	-------	.1699	.088	-------	-------	-------	-------
Lot 2. Length 7.2 to 7.8 mm. at time of gathering	.2346	.1780	.2443	-------	.2103	.13	-------	-------	-------	-------
	.2815	.2265	.2265	-------	-------	.11	-------	-------	-------	-------
	.2961	-------	-------	-------	-------	.11	-------	-------	-------	-------
	.1747	-------	-------	-------	-------	.09	-------	-------	-------	-------
	.1893	-------	-------	-------	-------	.10	-------	-------	-------	-------
Average	.2352	.2022	.2354	-------	.2103	.108	-------	-------	-------	-------

FROM HEADS ON CULM STORED WITHOUT DRYING

Under these conditions the culms and leaves remained almost turgid and the kernels showed no signs of water loss until the sixth day when they were first noticeably flaccid. A single kernel was picked from each head and killed at the time the culm was taken from the plant, a similar kernel being picked and killed every second day thereafter. These kernels later were sectioned. Camera-lucida drawings of the embryos treated on the successive dates are shown in Figure 1. These drawings show a marked increase in size

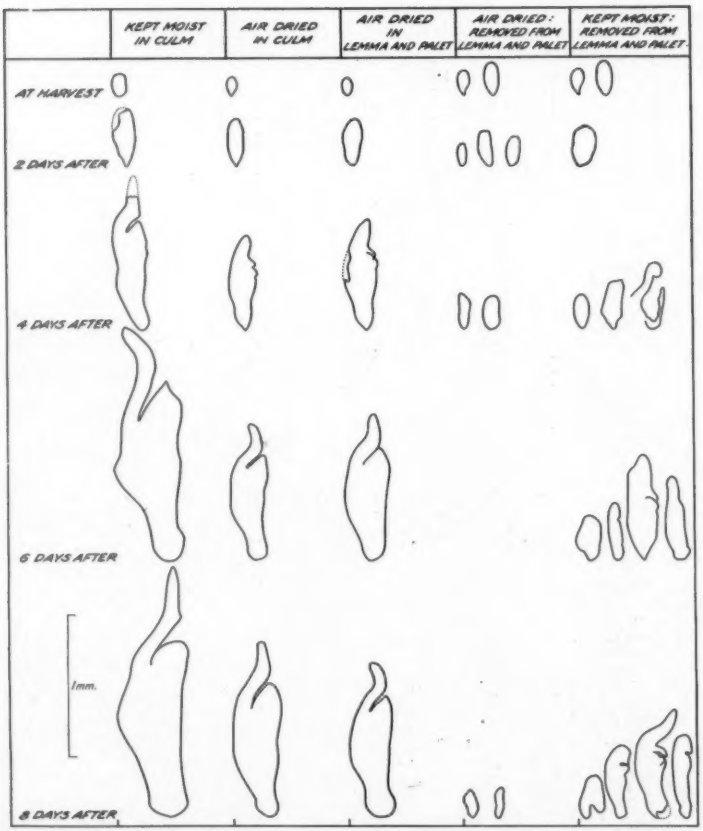


FIG. 1.—Growth in immature barley embryos resulting from different treatments after sampling

and in differentiation of tissue with each successive sampling. At the time the culm was gathered the embryo was a mass of cells, generally oblately spheroidal in section, and with no differentiation of parts. By the second day a marked polarity became visible because of more rapid growth at the distal end. On the fourth day

the scutellum was well marked and the "analgen" of radicle and plumule became apparent. On the sixth day the various embryo parts were still further developed.

From Heads on Culm, Air-Dried

The foliage rapidly lost water and became brittle. The kernels also lost water, but more slowly than did the foliage. They were shrunken but still green and pliable on the eighth day. The embryos of these heads did not grow quite as much as did those kept moist. Figures showing cell division were visible in the sections from all the five harvesting dates in both this and the preceding lots. Growth should not be confused with increase in weight, as elongation and differentiation of tissue can take place without increase in weight. Probably all three occur with some treatments.

Kernels Retaining Lemma and Palet Only, Air-Dried

Typical kernels ranged from 5.2 mm. to 5.8 mm. in length. Notwithstanding the drying, the kernels were still green and pliable on the eighth day, but of course were much shrunken from loss of water. The embryos apparently grew about the same amount as when the kernels were air-dried on the culm. Sections from two and four days after sampling showed mitotic division.

Naked Kernels, Air-Dried

They were still pliable on the fourth day and rather stiff but still tough enough to bend without breaking on the sixth day. Little, if any, embryo growth occurred in these kernels.

Naked Kernels Stored in a Moist Chamber

These kernels remained turgid, but became malformed. As shown in the drawings there seemed to have been a certain amount of growth, but it was not very consistent either with the age of the kernels or day of killing. However, in only 2 of the 11 cases was an embryo shorter than the average of the previous date of sampling. Inconsistency, however, as shown in the figure, is very marked. In many of these embryos the proximal end appeared to have disintegrated, and the embryo as a whole was almost as misshapen as the kernel. In such embryo tissue the cells seemed to have increased in size merely by the absorption of water. The malformed condition of the kernel was at first thought due to absorption of water from contact with the moist filter paper.

This part of the experiment was repeated in 1925, with a slight modification in that the kernels were placed on a dry glass plate elevated above a layer of wet cotton in the bottom of a Petri dish. This gave a moisture-saturated atmosphere without a contact which might affect the results. The kernels were still somewhat malformed, showing about the same characteristics as when laid on the wet filter paper.

DISCUSSION

These experiments have a bearing on several mooted points in the relation of kernel to plant. It is frequently assumed that the severing of the vascular system of a culm results in air bubbles in the conductive cells and promptly interrupts all communication with the kernel. It is a not uncommon belief that growth in the kernel is analogous to the growth process in other parts of the plant. On this latter point there would seem to be a considerable independence of development, both of the kernel with respect to the plant, and of the embryo with respect to the kernel. The embryo, for instance, lies in a lake of cell sap at the base of the nucellus. It obtains its nourishment by absorption, and as long as the body of sap is present and contains the elements for growth it should be of little moment to the embryo whether or not the culm is still connected with the plant.

Growth also is an indefinite term. Growth may consist of the elongation or distension of cells already present without the addition of new material other than water. It may consist of the adding of new cells from materials already present. It may be differentiation of tissue without new material, it may be development of embryo at the expense of endosperm, or it may be actual physical increase of material as well as increase in size and differentiation. It is obvious, from the results reported here, that differentiation can occur without increase in weight of kernel, as in the limited development of embryos in kernels dehulled and kept moist. The nucellus is surrounded by a thick pericarp, which, in young kernels, is turgid with water. Some of the cells even contain starch. It is not demonstrated that solid materials are received from the pericarp, but surely this tissue helps to maintain conditions favorable for the utilization of the cell sap within the nucellus by the embryo.

It also seems evident that the kernel is able to extract a limited amount of food material from the culm, even when this is severed and dried immediately in an arid atmosphere. Cell divisions and increase in weight are apparently demonstrated. Conceding this to be fact, it is probable that the changes that occur in the shock of grain harvested at maturity may be of importance.

SUMMARY AND CONCLUSIONS

Immature barley kernels kept moist on the culm after sampling will continue to grow, both in endosperm and in embryo, for at least eight days after harvest.

The embryos of immature kernels allowed to air-dry on the culm will continue to grow for at least eight days after sampling. The final length in millimeters, however, is approximately 40 per cent less than when the culm is kept moist.

The embryos of immature kernels dried in the glumes will grow about as much as those dried on the culm.

There is no evidence of growth in immature kernels if removed from the culm, separated from lemma and palet, and air-dried.

Where naked immature kernels are kept moist, their embryos may grow, but not consistently. The growth of such embryos must have occurred at the expense of the soluble food supply contained in the surrounding tissues.

The immature kernel almost certainly abstracts food material from the culm after harvest.

NUTRITIVE VALUE OF THE PROTEIN IN VEAL AND CALF SWEETBREADS; IN BEEF CHEEK MEAT, LIPS, TONGUES, BRAINS, SPLEEN, AND TRIPE; AND IN HOG BRAINS AND TONGUES [1]

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INTRODUCTION

Veal is the most important of the several products investigated in the work covered by this paper. The per capita consumption of veal in this country in 1924 was 8.3 pounds, as compared with 5.3 pounds for mutton and lamb.[2] The total amount of the other tissues produced in a year is considerable, but the per capita consumption is small.

Sweetbreads (the thymus glands of the calf), are a highly prized and relatively expensive meat product. Beef cheek meat and lips, which are trimmed from cattle heads at the time of slaughter, are used chiefly in the manufacture of sausage. Ox spleens are used to only a limited extent as food. A few are sold fresh and some are used in the manufacture of sausage. Tripe, which is prepared from the walls of the first and second stomachs of cattle, is sold either fresh or pickled, or it is used in the manufacture of sausage. Ox tongues are sold fresh, pickled, smoked, or canned, and hog tongues are usually sold either as jellied tongue or as canned lunch tongues. Ox and hog brains are sold fresh.

All the above-named products are of value for food chiefly on account of their protein, and it was the purpose of this investigation to determine the relative nutritive values of the proteins in the several tissues.

There seems to be very little information in the literature concerning the nutritive values of the proteins in the tissues under investigation. Mitchell[3] determined the biological value of the protein in veal and certain other food products by feeding tests with albino rats. When protein was fed at a 5 per cent level, veal had a value of 97 as compared with 94 for milk and 92 for beef. When protein was fed at a level of 8 to 10 per cent, veal had a value of 84 as compared with 83 for milk and 81 for beef. It appeared that the protein in veal had practically the same value as that in milk or beef.

EXPERIMENTAL WORK

PROCEDURE

In the experiments reported in this paper, the nutritive value of the protein in each tissue was determined by means of feeding

[1] Received for publication Oct. 9, 1925; issued March, 1926.

[2] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF ANIMAL INDUSTRY. MEAT PRODUCTION, CONSUMPTION, AND FOREIGN TRADE IN UNITED STATES, CALENDAR YEARS 1907–1924. Compiled by J. Roberts. 9 p. [1925?] [Mimeographed.]

[3] MITCHELL, H. H. THE PLACE OF PROTEINS IN THE DIET IN THE LIGHT OF THE NEWER KNOWLEDGE OF NUTRITION. Amer. Jour. Pub. Health 13: 17–23. 1923.

tests with young male albino rats.[4] The methods employed in this investigation are essentially the same as those followed by the writers in a previous study of the nutritive value of the protein in certain other animal tissues,[5] and they will not be described here.

With one exception, the products were purchased in fresh condition from local packinghouses. The ox lips were obtained in Chicago. The veal consisted of the forequarters from a good grade of veal calves. The muscle tissue was trimmed free from fat and connective tissue, ground, and dried in a current of air at a temperature approximating 60° C. The other tissues were trimmed free from extraneous material and dried in the same manner. All tests were conducted with the dried material. For comparison, the nutritive value of casein and of the combined proteins in milk was determined also. The casein was a highly purified product, and a well-known brand of dried, partially skimmed milk was the source of milk protein.

RESULTS OF 30-DAY FEEDING TESTS WITH RATIONS CONTAINING 10 PER CENT PROTEIN [6]

The detailed results of this series of experiments are presented in Table I, and the average results in Table II. In Table II are reported also the average values obtained for the protein in ox, hog, and sheep muscle in a previous investigation by the writers.[5] In the interpretation of the results of these experiments, the relation between the feed and protein intake, and the gain in weight of the rats, is of the greatest importance as an indication of the relative nutritive values of the protein in the several products. Gain in weight alone is not a correct basis for comparing the values of the various tissues as sources of protein.

On referring to Tables I and II, it appears that when protein was fed at a 10 per cent level of intake and the duration of the experiments was 30 days, the following products had approximately the same value as sources of protein for maintenance and growth in young albino rats, viz: Veal, ox brain, hog brain, ox tongue, hog tongue, ox spleen, ox muscle, hog muscle, sheep muscle, and milk.

Among these products, ox spleen had the lowest value with a gain in weight of 2.75 grams for each gram of protein consumed, and ox muscle had the highest value with a gain in weight of 3.15 grams; but it is doubtful if the difference between these values is large enough to be significant.

On the other hand, the protein in the following products appeared to be of distinctly lower nutritive value, viz: Sweetbreads, beef cheek meat, ox lips, tripe, and casein. Of these products, sweetbreads and tripe had the lowest values, beef cheek meat the highest value, and casein and ox lips occupied an intermediate position. The gain in weight for each gram of protein consumed ranged from 1.73 grams for tripe to 2.36 grams for beef cheek meat.

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[4] The writers are indebted to Warren G. Briggs, of the Biochemic Division, for assistance in carrying on the feeding tests with rats.

[5] HOAGLAND, R., and SNIDER, G. G. NUTRITIVE VALUE OF PROTEIN IN VOLUNTARY MUSCLE, HEART, LIVER, AND KIDNEY FROM CATTLE, SHEEP AND HOGS. [Unpublished manuscript.]

[6] In this paper the term "protein" refers to the result obtained by multiplying the nitrogen content of a product by the factor 6.25.

TABLE I.—*Nutritive value of protein in animal tissues when fed at the 10 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at start	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Veal	665A	28	41	90	295	29.5	3.3	0.33	0.31	3.05
Do	665B	28	44	79	273	27.3	3.5	.35	.29	2.90
Do	665C	28	40	57	197	19.7	3.5	.35	.29	2.90
Do	665D	28	44	56	187	18.7	3.3	.33	.30	2.99
Do	665E	28	44	83	284	28.4	3.4	.34	.29	2.92
Do	665F	28	41	51	201	20.1	4.0	.40	.25	2.53
Average		28	42	69	240	24.0	3.5	.35	.29	2.88
Ox brain	586A	26	44	94	305	30.5	3.2	.32	.31	3.08
Do	586B	26	39	86	288	28.8	3.3	.33	.30	2.99
Do	586C	24	40	68	248	24.8	3.6	.36	.27	2.74
Do	586D	24	43	71	253	25.3	3.6	.36	.28	2.81
Do	586E	25	45	99	315	31.5	3.2	.32	.31	3.14
Do	586F	25	38	80	261	26.1	3.3	.33	.31	3.07
Average		25	42	83	278	27.8	3.4	.34	.30	2.97
Hog brain	658A	27	44	79	264	26.4	3.3	.33	.30	2.99
Do	658B	27	40	79	243	24.3	3.1	.31	.33	3.25
Do	658C	27	39	56	184	18.4	3.3	.33	.30	3.00
Do	658D	27	38	48	179	17.9	3.7	.37	.27	2.68
Do	658E	30	41	72	245	24.5	3.4	.34	.29	2.93
Do	658F	30	42	62	248	24.8	4.0	.40	.25	2.50
Average		28	41	66	227	22.7	3.5	.35	.29	2.89
Ox tongue	671A	30	43	67	276	27.6	4.1	.41	.24	2.43
Do	671B	30	38	68	237	23.7	3.5	.35	.29	2.87
Do	671C	29	45	77	231	23.1	3.0	.30	.33	3.33
Do	671D	29	39	86	296	29.6	3.4	.34	.29	2.90
Do	671E	26	41	83	252	25.2	3.0	.30	.33	3.29
Do	671F	26	38	62	275	27.5	4.4	.44	.23	2.26
Average		28	41	74	261	26.1	3.6	.36	.29	2.85
Hog tongue	672A	26	39	93	367	36.7	3.9	.39	.25	2.53
Do	672B	29	44	107	363	36.3	3.4	.34	.29	2.95
Do	672C	29	42	73	249	24.9	3.4	.34	.29	2.93
Do	672D	29	41	86	282	28.2	3.3	.33	.30	3.00
Do	672E	28	39	102	324	32.4	3.2	.32	.31	3.15
Do	672F	28	41	82	315	31.5	3.8	.38	.26	2.60
Average		28	41	91	316	31.6	3.5	.35	.28	2.86
Calf sweetbreads (thymus gland)	576A	25	39	36	188	18.8	5.2	.52	.19	1.91
Do	576B	27	42	31	163	16.3	5.3	.53	.19	1.90
Do	576C	27	45	31	186	18.6	6.0	.60	.17	1.67
Do	576D	27	41	23	150	15.0	6.5	.65	.15	1.53
Do	576E	29	45	40	212	21.2	5.3	.53	.19	1.89
Do	576F	30	39	32	164	16.4	5.1	.51	.20	1.95
Average		28	42	32	177	17.7	5.6	.56	.18	1.81
Beef cheek meat	673A	28	42	45	181	18.1	4.0	.40	.25	2.49
Do	673B	29	38	68	238	23.8	3.5	.35	.29	2.86
Do	673C	30	44	55	230	23.0	4.2	.42	.24	2.39
Do	673D	30	40	52	223	22.3	4.3	.43	.23	2.33
Do	673E	30	43	43	224	22.4	5.2	.52	.19	1.92
Do	673F	30	39	50	229	22.9	4.6	.46	.22	2.18
Average		30	41	52	221	22.1	4.3	.43	.24	2.36
Ox lips	676A	30	41	57	234	23.4	4.1	.41	.24	2.44
Do	676B	30	40	44	202	20.2	4.6	.46	.22	2.17
Do	676D	30	45	51	229	22.9	4.5	.45	.22	2.23
Do	676E	30	45	57	251	25.1	4.4	.44	.23	2.27
Do	676F	27	42	53	224	22.4	4.2	.42	.24	2.36
Average		29	43	52	228	22.8	4.4	.44	.23	2.29

TABLE I.—*Nutritive value of protein in animal tissues when fed at the 10 per cent level for 30 days to young male albino rats*—Continued

Source of protein	Rat No.	Age at start	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Tripe	616A	27	39	30	150	15.0	5.0	.50	.20	2.00
Do	616B	27	39	27	170	17.0	6.3	.63	.16	1.60
Do	616C	28	41	31	167	16.7	5.4	.54	.19	1.86
Do	616D	25	42	30	159	15.9	5.3	.53	.19	1.88
Do	616E	23	39	28	138	13.8	4.9	.49	.20	2.03
Do	616F	23	39	11	107	10.7	9.7	.97	.10	1.03
Average		26	40	26	149	14.9	6.1	.61	.17	1.73
Ox spleen	593A	26	39	63	233	23.3	3.7	.37	.27	2.70
Do	593B	28	43	92	303	30.3	3.3	.33	.30	3.03
Do	593C	27	42	74	256	25.6	3.5	.35	.29	2.89
Do	593D	27	39	60	219	21.9	3.7	.37	.27	2.74
Do	593E	27	45	80	298	29.8	3.7	.37	.27	2.68
Do	593F	27	39	61	248	24.8	4.1	.41	.25	2.46
Average		27	41	72	260	26.0	3.7	.37	.28	2.75
Casein	667A	29	43	25	139	13.9	5.6	.56	.18	1.80
Do	667B	29	43	35	194	19.4	5.5	.55	.18	1.80
Do	667C	30	39	27	147	14.7	5.4	.54	.18	1.84
Do	667D	25	40	26	133	13.3	5.1	.51	.20	1.95
Do	667E	25	41	48	205	20.5	4.3	.43	.23	2.34
Do	667F	25	39	39	183	18.2	4.7	.47	.21	2.14
Average		27	41	33	167	16.7	5.1	.51	.20	1.98
Milk	621A	26	40	61	196	19.6	3.2	.32	.31	3.11
Do	621B	25	41	64	223	22.3	3.5	.35	.29	2.87
Do	621C	26	44	69	255	25.5	3.7	.37	.27	2.71
Do	621D	28	43	57	259	25.9	4.5	.45	.22	2.20
Do	621E	22	38	86	247	24.7	2.9	.29	.35	3.48
Do	621F	30	38	63	212	21.2	3.4	.34	.30	2.97
Average		26	41	67	232	23.2	3.5	.35	.29	2.89

TABLE II.—*Average nutritive values obtained for protein in animal tissues when fed at the 10 per cent level for 30 days to young male albino rats.*

Source of protein	Number of rats	Age at start	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Veal	6	28	42	69	240	24.0	3.5	0.35	0.29	2.88
Ox brain	6	25	42	83	278	27.8	3.4	.34	.30	2.97
Hog brain	6	28	41	66	227	22.7	3.5	.35	.29	2.89
Ox tongue	6	29	41	74	261	26.1	3.6	.36	.29	2.85
Hog tongue	6	28	41	91	316	31.6	3.5	.35	.28	2.86
Thymus (sweetbread)	6	28	42	32	177	17.7	5.6	.56	.18	1.81
Beef, cheek meat	6	30	41	52	221	22.1	4.3	.43	.24	2.36
Ox lips	5	29	43	52	228	22.8	4.4	.44	.23	2.29
Tripe	6	26	40	26	149	14.9	6.1	.61	.17	1.73
Ox spleen	6	27	41	72	260	26.0	3.7	.37	.28	2.75
Casein	6	27	41	33	167	16.7	5.1	.51	.20	1.98
Milk	6	26	41	67	232	23.2	3.5	.35	.29	2.89
Ox muscle *	4	31	47	95	302	30.2	3.2	.32	.31	3.15
Hog muscle *	4	24	44	84	281	28.1	3.4	.34	.30	2.96
Sheep muscle *	4	24	41	85	274	27.4	3.2	.32	.31	3.12

* HOAGLAND, R., and SNIDER, G. G. NUTRITIVE VALUE OF PROTEIN IN VOLUNTARY MUSCLE, HEART, LIVER, AND KIDNEY FROM CATTLE, SHEEP, AND HOGS. [Unpublished manuscript.]

RESULTS OF 60-DAY FEEDING TESTS WITH RATIONS CONTAINING 10 PER CENT PROTEIN

The results of these experiments are reported in Tables III and IV. These tests, which are a continuation of those reported in Tables I and II, covered a total period of 60 days. Casein and tripe were not tested for the 60-day period.

Referring to Tables III and IV, there may be observed the same general differences in the nutritive values of the proteins in the various products as were noted in the 30-day experiments, but the differences are not so pronounced for the longer period as they were for the shorter one. It appears that veal, ox brains, hog brains, ox tongues, hog tongues, ox spleens, ox muscle, hog muscle, sheep muscle, and milk had practically the same value as sources of protein, and that beef cheek meat had a slightly lower value. Ox lips and sweetbreads, on the other hand, had decidedly lower protein values, although the differences are not as great as they were in the 30-day experiments.

TABLE III.—*Nutritive value of protein in animal tissues when fed at 10 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at start	Initial weight	Gain in weight 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Veal	665A	28	41	173	715	71.5	4.1	0.41	0.24	2.42
Do	665B	28	44	144	629	62.9	4.4	.44	.23	2.29
Do	665C	28	40	125	522	52.2	4.2	.42	.24	2.39
Do	665E	28	44	141	629	62.9	4.5	.45	.22	2.24
Do	665F	28	41	122	505	50.5	4.1	.41	.24	2.42
Average		28	42	141	600	60.0	4.3	.43	.23	2.35
Ox brain	586A	26	44	171	678	67.8	4.0	.40	.25	2.52
Do	586B	26	39	161	637	63.7	4.0	.40	.25	2.53
Do	586C	24	40	153	597	59.7	3.9	.39	.26	2.56
Do	586D	24	43	150	579	57.9	3.9	.39	.26	2.59
Do	586E	25	45	168	728	72.8	4.3	.43	.23	2.31
Do	586F	25	38	138	616	61.6	4.5	.45	.22	2.24
Average		25	42	157	639	63.9	4.1	.41	.25	2.46
Hog brain	658A	27	44	140	625	62.5	4.5	.45	.22	2.24
Do	658B	27	40	147	598	59.8	4.1	.41	.25	2.46
Do	658C	27	39	105	468	46.8	4.5	.45	.22	2.24
Do	658D	27	38	102	447	44.7	4.4	.44	.23	2.28
Do	658E	30	41	153	613	61.3	4.0	.40	.25	2.50
Do	658F	30	42	129	587	58.7	4.6	.46	.22	2.20
Average		28	41	129	556	55.6	4.4	.44	.23	2.32
Ox tongue	671A	30	43	129	634	63.4	4.9	.49	.20	2.03
Do	671B	30	38	132	557	55.7	4.2	.42	.24	2.37
Do	671C	29	45	118	500	50.0	4.2	.42	.24	2.36
Do	671D	29	39	154	648	64.8	4.2	.42	.24	2.38
Do	671E	26	41	135	534	53.4	3.9	.39	.25	2.53
Do	671F	26	38	112	551	55.1	4.9	.49	.20	2.03
Average		28	41	130	571	57.1	4.4	.44	23	2.28
Hog tongue	672A	26	39	160	789	78.9	4.9	.49	.20	2.03
Do	672B	29	44	196	808	80.8	4.1	.41	.24	2.42
Do	672C	29	42	112	539	53.9	4.8	.48	.21	2.08
Do	672D	29	41	148	645	64.5	4.4	.44	.23	2.29
Do	672E	28	39	169	672	67.2	4.0	.40	.25	2.51
Do	672F	28	41	163	739	73.9	4.5	.45	.22	2.21
Average		28	41	158	699	69.9	4.5	.45	.23	2.26

TABLE III.—*Nutritive value of protein in animal tissues when fed at 10 per cent level for 60 days to young male albino rats*—Continued

Source of protein	Rat No.	Age at start	Initial weight	Gain in weight 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Calf sweetbreads (thymus gland)	576A	25	39	73	429	42.9	5.9	.59	.17	1.70
Do	576B	25	42	70	381	38.1	5.4	.54	.18	1.84
Do	576C	27	45	67	405	40.5	6.0	.60	.16	1.65
Do	576D	27	41	60	374	37.4	6.2	.62	.16	1.60
Do	576E	25	45	59	414	41.4	7.0	.70	.14	1.42
Do	576F	30	39	63	396	39.6	6.3	.63	.16	1.59
Average		27	42	65	400	40.0	6.1	.61	.16	1.63
Beef cheek meat	673A	28	42	101	446	44.6	4.4	.44	.23	2.27
Do	673B	29	38	101	511	51.1	5.1	.51	.19	1.98
Do	673C	30	44	110	549	54.9	5.0	.50	.20	2.00
Do	673D	30	40	113	499	49.9	4.4	.44	.23	2.26
Do	673E	30	43	101	488	48.8	4.8	.48	.21	2.07
Do	673F	30	39	112	509	50.9	4.5	.45	.22	2.20
Average		29	41	106	500	50.0	4.7	.47	.21	2.13
Ox lips	676A	30	41	87	495	49.5	5.7	.57	.18	1.76
Do	676B	30	40	86	430	43.0	5.0	.50	.20	2.00
Do	676D	30	45	78	463	46.3	5.9	.59	.17	1.69
Do	676E	30	45	100	539	53.9	5.4	.54	.19	1.86
Do	676F	27	42	80	457	45.7	5.7	.57	.18	1.75
Average		29	43	86	477	47.7	5.5	.55	.18	1.81
Ox spleen	593A	26	39	135	555	55.5	4.1	.41	.24	2.43
Do	593B	28	43	172	654	65.4	3.8	.38	.26	2.63
Do	593C	27	42	147	586	58.6	4.0	.40	.25	2.51
Do	593D	27	39	122	513	51.3	4.2	.42	.24	2.38
Do	593E	27	45	117	591	59.1	5.1	.51	.20	1.98
Do	593F	27	39	123	593	59.3	4.8	.48	.21	2.07
Averafe		27	41	136	582	58.2	4.3	.43	.23	2.33
Milk	621A	26	40	117	467	46.7	4.0	.40	.25	2.50
Do	621B	25	41	133	520	52.0	3.9	.39	.26	2.56
Do	621C	26	44	114	530	53.0	4.6	.46	.22	2.15
Do	621D	28	43	102	543	54.3	5.3	.53	.19	1.88
Do	621E	22	38	149	569	56.9	3.8	.38	.26	2.62
Do	621F	30	38	122	499	49.9	4.1	.41	.24	2.44
Average		26	41	123	521	51.1	4.3	.43	.24	2.36

TABLE IV.—*Average nutritive values obtained for protein in animal tissues when fed at the 10 per cent level for 60 days to young male albino rats*

Source of protein	Number of rats	Age at start	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protien
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Veal	5	28	42	141	600	60.0	4.3	0.43	0.23	2.35
Ox brain	6	25	42	157	639	63.9	4.1	.41	.25	2.46
Hog brain	6	28	41	129	556	55.6	4.4	.44	.23	2.32
Ox tongue	6	28	41	130	571	57.1	4.4	.44	.23	2.28
Hog tongue	6	28	41	158	699	69.9	4.5	.45	.23	2.26
Thymus	6	28	42	65	400	40.0	6.1	.61	.16	1.63
Beef cheek meat	6	30	41	106	500	50.0	4.7	.47	.21	2.13
Ox lips	5	29	43	86	477	47.7	5.5	.55	.18	1.81
Ox spleen	6	27	41	136	582	58.2	4.3	.43	.23	2.33
Milk	6	26	41	123	521	52.1	4.3	.43	.24	2.36
Ox muscle [a]	4	31	47	170	667	66.7	3.9	.39	.25	2.55
Hog muscle [a]	4	24	44	137	554	55.4	4.1	.41	.25	2.46
Sheep muscle [a]	4	24	41	152	611	61.1	4.1	.41	.25	2.48

[a] HOAGLAND, R., and SNIDER, G. G., NUTRITIVE VALUE OF PROTEIN IN VOLUNTARY MUSCLE, HEART, LIVER, AND KIDNEY FROM CATTLE, SHEEP, AND HOGS. [Unpublished manuscript.]

RESULTS OF 30-DAY FEEDING TESTS WITH RATIONS CONTAINING 12.5 PER CENT PROTEIN

Only a few of the tissues previously tested were fed in rations containing 12.5 per cent protein for the 30-day period. The results of these experiments, together with data previously obtained for ox and hog muscle, are reported in Table V. It will be noted again, as in the experiments with the rations containing 10 per cent protein, that the protein in veal has practically the same nutritive value as that in ox and hog muscle; that the beef cheek meat and ox lips have considerably lower protein values, and that tripe protein has the lowest value of all. Comparing the data in Table V with those in Table I, the rats fed the ration containing 12.5 per cent tripe protein for a period of 30 days made slightly less growth than those fed the ration containing 10 per cent of tripe protein for the same length of time.

TABLE V.—*Nutritive value of protein in animal tissues when fed at the 12.5 per cent level for 30 days to young male albino rats*

Source of protein	Rat No.	Age at start	Initial weight	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Veal	666A	29	42	97	269	33.6	2.8	0.35	0.36	2.89
Do	666B	29	45	109	305	38.1	2.8	.35	.36	2.86
Do	666C	29	40	107	289	36.1	2.7	.34	.37	2.96
Do	666D	29	40	92	260	32.5	2.8	.35	.35	2.83
Do	666E	29	40	93	257	32.1	2.8	.35	.32	2.90
Do	666F	29	39	102	275	34.4	2.6	.34	.37	2.97
Average		29	41	100	276	34.5	2.8	.35	.36	2.90
Beef cheek meat	674A	30	38	76	263	32.9	3.5	.43	.29	2.31
Do	674B	30	42	82	236	29.5	2.9	.36	.35	2.78
Do	674C	29	41	72	250	31.3	3.5	.43	.29	2.30
Do	674D	29	43	98	308	38.5	3.1	.39	.32	2.55
Do	674E	29	39	84	270	33.8	3.2	.40	.31	2.49
Do	674F	29	39	121	330	41.3	2.7	.34	.37	2.93
Average		29	40	89	276	34.6	3.2	.39	.32	2.56
Ox lips	677A	27	38	70	237	29.6	3.4	.42	.29	2.37
Do	677B	28	38	57	205	25.6	3.6	.45	.28	2.23
Do	677C	28	39	61	197	24.6	3.2	.40	.31	2.48
Do	677D	24	38	70	261	32.6	3.7	.47	.27	2.15
Do	677E	24	40	104	310	38.8	3.0	.37	.34	2.68
Do	677F	23	42	104	330	41.3	3.2	.40	.32	2.52
Average		26	39	78	257	32.1	3.4	.42	.30	2.41
Tripe	628A	28	41	20	133	16.6	6.7	.83	.15	1.20
Do	628B	24	40	29	153	19.1	5.3	.66	.19	1.52
Do	628C	24	39	14	127	15.9	9.1	1.14	.11	.88
Do	628D	24	38	15	117	14.6	7.8	.97	.13	1.03
Average		25	40	20	133	16.6	7.2	.90	.15	1.16
Ox muscle *		30	50	107	313	39.1	2.9	.37	.34	2.73
Hog muscle *		27	43	108	308	38.5	2.9	.36	.35	2.82

* HOAGLAND, R., and SNIDER, G. G. NUTRITIVE VALUE OF PROTEIN IN VOLUNTARY MUSCLE, HEART, LIVER, AND KIDNEY FROM CATTLE, SHEEP AND HOGS. [Unpublished manuscript.]

RESULTS OF 60 DAY FEEDING TESTS WITH RATIONS CONTAINING 12.5 PER CENT PROTEIN

Only veal, beef cheek meat and ox lips were fed in rations containing 12.5 per cent protein for the 60-day period. The results of these tests, together with those previously obtained for ox and hog muscle, are presented in Table VI. These data indicate relatively small differences in the nutritive values of the protein in the several tissues, although beef cheek meat and ox lips have slightly lower values than the other tissues.

TABLE VI.—*Nutritive value of protein in animal tissues when fed at the 12.5 per cent level for 60 days to young male albino rats*

Source of protein	Rat No.	Age at start	Initial weight	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
					Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Veal	666A	29	42	157	592	74.0	3.7	0.47	0.27	2.15
Do	666B	29	45	172	680	85.0	3.9	.49	.25	2.02
Do	666C	29	40	169	638	79.8	3.8	.48	.26	2.12
Do	666D	29	40	141	570	71.3	4.0	.51	.25	1.98
Do	666E	29	40	163	623	77.9	3.8	.48	.26	2.09
Do	666F	29	39	177	628	78.5	3.5	.44	.28	2.25
Average		29	41	163	622	77.8	3.8	.48	.26	2.10
Beef cheek meat	674A	30	38	137	590	73.8	4.3	.54	.23	1.86
Do	674B	30	42	143	550	68.8	3.8	.48	.26	2.08
Do	674C	29	41	147	560	70.0	3.8	.48	.26	2.10
Do	674D	29	43	158	647	80.9	4.1	.51	.24	1.95
Do	674E	29	39	155	625	78.1	4.0	.50	.25	1.98
Do	674F	29	39	190	706	88.3	3.7	.46	.27	2.15
Average		29	40	155	613	76.7	4.0	.49	.25	2.02
Ox lips	677A	27	38	124	534	66.8	4.3	.54	.23	1.86
Do	677B	28	38	112	435	54.4	3.9	.49	.26	2.06
Do	677C	28	39	120	487	60.9	4.1	.51	.25	1.97
Do	677D	24	38	138	643	80.4	4.7	.58	.21	1.72
Do	677E	24	40	161	663	82.9	4.1	.51	.24	1.94
Do	677F	23	42	193	748	93.5	3.9	.48	.26	2.06
Average		26	39	141	585	73.2	4.2	.52	.24	1.94
Ox muscle[a]		30	50	194	718	89.8	3.8	.47	.26	2.14
Hog muscle[a]		27	43	174	668	83.5	3.9	.49	.26	2.08

[a] HOAGLAND, R., and SNIDER, G. G. NUTRITIVE VALUE OF PROTEIN IN VOLUNTARY MUSCLE, HEART, LIVER, AND KIDNEY FROM CATTLE, SHEEP AND HOGS. [Unpublished manuscript.]

RESULTS OF 30 AND 60 DAY FEEDING TESTS WITH RATIONS CONTAINING 20 PER CENT OF TRIPE PROTEIN

The reults of these experiments are reported in Table VII. Somewhat better results were obtained with the ration containing 20 per cent tripe protein than had been obtained previously with rations containing smaller proportions of this protein, but growth was still subnormal and irregular. Referring to Table VII, it will be noted for the rats which were fed tripe for the 60-day period, that the gains in weight of the 4 rats were as follows: 18, 30, 90, and 104 grams, average 61 grams; whereas the rats which were fed the ration containing 12.5 per cent veal protein for the same period (Table VI) made gains in weight ranging from 141 to 177 grams, average 163 grams.

TABLE VII.—*Nutritive value of protein in tripe when fed at the 20 per cent level for 30 and 60 days, respectively, to young male albino rats*

Source of protein	Rat No.	Age at start	Initial weight	Duration of test	Gain in weight	Total intake		Intake per gram gain in weight		Gain in weight	
						Feed	Protein	Feed	Protein	Per gram feed	Per gram protein
		Days	*Grams*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Tripe	655 A	28	45	30	37	148	29.6	4.0	0.80	0.25	1.25
Do	655 B	28	38	30	33	131	26.2	4.0	.80	.25	1.26
Do	655 C	26	39	30	20	108	21.6	5.4	1.08	.19	.93
Do	655 D	25	38	30	56	173	34.6	3.1	.62	.32	1.62
Average		27	40	30	37	140	28.0	4.1	.83	.25	1.27
Tripe	655 A	28	45	60	90	357	71.4	4.0	.79	.25	1.26
Do	655 B	28	38	60	30	243	48.6	8.1	1.62	.12	.62
Do	655 C	26	39	60	18	200	40.0	11.1	2.22	.09	.45
Do	655 D	25	38	60	104	381	76.2	3.7	.73	.27	1.36
Average		27	40	60	61	295	59.1	6.7	1.34	.18	.92

DISCUSSION AND SUMMARY

In this paper are reported the results of a study of the nutritive value of a number of animal tissues as sources of protein for maintenance and growth in young male albino rats.

Protein was fed at three levels of intake, viz: 10, 12.5, and 20 per cent, but most of the products were tested at the lowest level, since it has been found that 10 per cent of an animal protein of good quality is somewhat less than is required for optimal growth in rats during a period of 30 to 60 days.

It was found that the protein in the following products had practically the same nutritive value when fed in rations containing 10 per cent protein for periods of 30 and 60 days, respectively: Veal, ox and hog brains, ox and hog tongues, ox spleens, and dried milk. The values obtained for these products agree very closely with those previously obtained by the writers for a number of other animal tissues, including ox, hog, and sheep muscle.

The proteins in the following products were found to have distinctly lower values when fed at the 10 per cent level for periods of 30 and 60 days, respectively: Sweetbreads, tripe, beef cheek meat, ox lips, and casein.

When protein was fed at a 12.5 per cent level for periods of 30 and 60 days, respectively, veal was found to have a somewhat higher value than beef cheek meat or ox lips, and practically the same value as had previously been obtained for ox and hog muscle.

The protein in tripe, even when fed at a 20 per cent level, was far from adequate for optimal growth in rats.

The poor quality of the protein in sweetbreads and tripe is presumably due to a deficiency of one or more of the essential amino acids, but information as to the nature of these deficiencies is lacking. The low nutritive value of the protein in beef cheek and ox lips is undoubtedly due to the presence of considerable connective tissue, which is known to be lacking in several essential amino acids. The low value of casein, when fed at the 10 per cent level, is due to

its well-known deficiency in cystine. This deficiency is made up, however, when casein is fed at a level of 18 to 20 per cent, and optimal growth then results.

Although the proteins in sweetbreads, tripe, beef cheek meat, and ox lips were found to be of relatively poor quality when any one of these products constituted the only source of protein in the diet, it is possible that the deficiencies of these proteins may be made up when they are fed in combination with other animal or vegetable proteins.

OBSERVATIONS ON A RECURRING OUTBREAK OF HETEROCAMPA GUTTIVITTA WALKER AND NATURAL ENEMIES CONTROLLING IT[1]

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INTRODUCTION

From 1907 to 1909 *Heterocampa guttivitta* Walk., a pest of forest and shade trees, became exceedingly abundant in the White Mountain region of New Hampshire and in some counties of western Maine. Much of the hardwood forest growth over many miles of territory was seriously or completely defoliated one or more times during that period. Particularly severe injury was suffered by the beech and sugar maple. Serious damage to forest growth was also reported from New York, Massachusetts, and Vermont. In 1909 W. F. Fiske and A. F. Burgess, of the Bureau of Entomology of the United States Department of Agriculture, begun a study of the natural control of the insect, continuing their investigations through the year 1910 (*2*, *5*).[2] Edith M. Patch (*7*), of the Maine Agricultural Experiment Station, and others have also published reports of their observations of the natural enemies of the pest during these outbreaks.

In 1917, 10 years after the beginning of the first infestation, *Heterocampa guttivitta* reappeared in damaging numbers in Litchfield County, Conn. (*1*), and in greater numbers through most of the beech and maple growth of the Berkshire Hills section of western Massachusetts (*4*). During August, 1918, the writer visited some sections of Massachusetts where sugar maple and beech grow abundantly, for the purpose of making observations on the extent of the infestation and damage to these trees and obtaining information concerning the natural enemies present.

DISTRIBUTION

Heterocampa guttivitta, apparently indigenous to North America, was first described by Walker in 1855 as *Cecrita guttivitta* (*8*, *p. 992*). Mention has been made of it in the literature under no fewer than five genera and seven species. It ranges from Canada through New England to Florida and Texas on the south and to Nebraska and Colorado on the west. Packard (*6*) in 1895 wrote that "two specimens only have been recorded from any of the New England States, where others will probably be discovered, though rarely.

[1] Received for publication Aug. 18, 1925; issued March, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 699.

It seems to abound most from Pennsylvania southward to Georgia." In going through the literature there seems to be no mention of the species having occurred in great abundance in any locality previous to 1907. Until then it was not of sufficient importance even to receive mention in Insect Life. In the early publications examined by the writer there are no records of the natural enemies of the species, but the data collected from 1907 to 1909, and those recorded here, indicate that conditions have greatly changed.

LIFE HISTORY

In New England, *Heterocampa guttivitta* has but one brood. The parent moths emerge in late May and early June. The moth has an expanse of about 2 inches; it is brownish gray in color, and the front wings are crossed by indistinct darker lines. Oviposition begins soon after emergence and copulation. The eggs, pale green in color, are deposited singly on beech, maple, and other hardwood leaves, and hatch in about nine days. The larvae become full grown in five weeks or more, according to weather conditions and food supply. In the first instar they bear nine pairs of black horns, the first pair of which, situated back of the head, are much longer than the others and branched like antlers. They vary in color from red and pink in the first two instars to green in the later ones. The larvae molt four times, and at the end of the last instar they enter the leaf mold or the ground beneath for pupation. Pupation occurs from the middle of July until late August, the maximum number of larvae entering the leaf mold late in July. The insect hibernates in the pupal stage in and under the leaf mold, the moths emerging the following spring.

RECENT OUTBREAKS

In order to estimate the injury caused to the trees after one or successive defoliations, some locations were selected and observations were made in them for a few years, and wood-lot owners in the infested district were consulted. In New England, defoliation by *Heterocampa guttivitta* occurs from early in July until August. The trees partly refoliate the same season, the quantity and size of the new foliage depending upon moisture conditions. Not more than two successive defoliations of 80 to 100 per cent severity were noted by the writer in the same area of beech or sugar maple. Some reports, however, were received from residents stating that as many as three successive annual defoliations occurred in the same woodland. In some woodlands where individual trees are subjected to repeated severe defoliations, coupled possibly with dry seasons in some cases and attacks by secondary insects in others, it is probable that these trees would lose some of their branches or succumb entirely. It is not known what part secondary insects played in these injuries, as time did not permit the investigation of such details.

The following towns in Massachusetts were visited in September, 1918: Worthington, Cummington, Hawley, Heath, Colraine, Ashfield, Plainfield, Charlemont, Middlefield, Becket, and Leyden. Many thousands of acres of beech growth intermingled with sugar maple, birch, and other hardwoods were affected in this section,

being wholly or partly defoliated. The centers of large areas were almost totally defoliated, and these areas were surrounded by others in varying stages of defoliation. The tier of towns to the west of those mentioned were almost as severely affected, according to reports received at the time, the infestation including sections of Hampshire, Franklin, and Berkshire Counties. Some damage was also noted in towns in southern Vermont near the Massachusetts boundary. In visiting the Massachusetts section in July and again in August, 1919, in company with Mr. Burgess, the writer observed many woodlands showing only 15 to 25 per cent defoliation, and a few more, composed of small areas, showing 60 to 70 per cent, which in 1918 had shown 90 to 100 per cent. Defoliation had been checked by natural enemies, as will be shown later. Observations in the same territory in 1920 resulted in the finding of scarcely any larvae.

Here and there cases were noted where complete or almost complete defoliation had occurred in two or more successive years. Howard Johnson, of Worthington, Mass., stated that many of his 2,700 sugar maple trees were entirely defoliated in 1917 and that about 200 of these did not refoliate in 1918. He also stated that he had made 5,600 pounds of sugar in 1917 but could make only 4,000 pounds in 1918, the decline in the yield of sugar being largely attributable to the injury caused by *Heterocampa guttivitta* the previous year. Where sugar-maple orchards were interspersed with beech or were situated on the edge of beech groves, the injury was greater to the maples, large trees or the larger limbs in some cases being killed. Where solid stands of beech suffered defoliation during two successive years, the damage was usually less severe than to maples in solid stands, for fewer of the large branches of the former die as a result of severe infestations. The injury to beech was apparent in the increased number of small branches killed and the putting out of undersized, yellowish-green, crumpled leaves throughout the tops of the trees, giving them a sickly and stunted appearance.

One large beech and sugar maple wood lot in Worthington, Mass., noted by the writer in 1918 as 90 to 100 per cent defoliated, was, according to property owners in the neighborhood, as badly defoliated in 1917. The same woodland in 1919 was only 15 to 20 per cent defoliated, but it was estimated that one-third of the beeches and one-half of the sugar maples around the edge of this grove had died and many branches and large portions of tops on others were dead. It was reported by one resident of a neighboring town that this area was also very badly infested in 1916, a fact which would partly explain the condition of the trees. The damage observed elsewhere in Massachusetts and New Hampshire was not nearly as severe as that reported above.

The writer was not able to make detailed observations in all parts of the area of severe infestation, but sufficient territory was examined to furnish what is believed to be a conservative estimate of the damage, namely, that the susceptible tree growth in areas upwards of 600 square miles in extent in both the western section of Massachusetts and the White Mountain section of New Hampshire where beech, sugar maple, and other hardwoods abound, suffered varying stages of defoliation from this insect between 1917 and 1920.

It was thought that *Anisota rubicunda* Fab., which was always present in small numbers, was responsible in a measure for the killing of the maple trees, but many examinations of the leaf mold late in the season always showed a very small percentage of these pupae compared to those of *Heterocampa guttivitta*. It was evident that the presence of the former species only supplemented the depredations of the latter in a small way.

In 1918 a report was received that *H. guttivitta* was increasing rapidly in Tamworth, N. H., and in September of that year observations were made there and in neighboring towns. One wood lot, mostly of beech (with some sugar maple and paper birch), was observed in which the defoliation was from 5 to 10 per cent, yet only 33 pupae were found in 12 square yards of leaf mold. Other woodlands were examined in towns near by which showed even smaller percentages of defoliation but as many pupae per square yard. The writer also visited woodlands in Denmark and East Denmark, Me., which had been defoliated two consecutive years in the previous outbreak, but he was unable to find pupae or signs of feeding on the beech leaves. A few dead-topped beeches and occasionally some dead branches were noted, but the trees and foliage, on the whole, were in a very thrifty condition.

Considering the slight defoliation evident in 1918 between the towns of Tamworth and Jackson, N. H., the writer was greatly surprised when he visited the same territory in 1919. The infestation had increased to such an extent that may thousands of acres of beech and intermingling hardwoods on the mountain sides showed from 90 to 100 per cent defoliation, each being surrounded by extensive areas in various stages of defoliation. Practically the same areas in this territory suffered as complete defoliation in 1920, although, if anything, the territory was slightly enlarged, for the depredations extended somewhat farther north, south, and east.

The climax of the infestation and injury to the forests was reached in New Hampshire in 1920. The phenomenal disappearance of the insects was noted in 1921, when, after diligent search in many locations, the writer was able to obtain only a very small number of larvae for rearing purposes. Maximum infestation was reached in the Massachusetts territory one year earlier, the last heavy defoliation having occurred there in 1919, but this was not nearly so heavy or extensive as that in 1918.

In June, 1922, examinations were again made for eggs and small larvae in parts of the area previously infested in Massachusetts and New Hampshire, but neither was found in the former section and only four eggs and no larvae in the latter. These efforts were repeated in the New Hampshire section in June, 1923, but only three eggs and no larvae were found. Attempts have been made to collect larvae annually since the outbreak in the New Hampshire section up to and including 1925, and in 1924 and 1925 in Massachusetts, with the result that only two were found and these were found in the latter section, in 1925.

NATURAL CONTROL [3]

Several visits were made to the infested region in search of data on natural enemies. Collections were made of the eggs for records on egg parasitism and of the larvae (both of the small and the more mature stages), and also of the pupae during the early fall for rearing and for determination by dissection of the various parasites and predators attacking this host. The larvae proved rather difficult to rear, many dying in confinement. Further difficulties were experienced in attempting to find suitable conditions under which the pupae would hibernate successfully and give normal emergence records of both adults and parasites. Consequently, it was thought best to dissect many of the pupae as soon as they were collected, and this made it difficult to determine the tachinids in their immature stages. Percentages of parasitism are therefore avoided in most cases in this paper.

To obtain the data on the natural enemies of the pupae, leaf mold was examined in selected locations, and all pupae found were taken for rearing or dissection or to determine the cause of death. Usually from 8 to 25 square yards of leaf mold were worked over in each location. On account of the necessity of continuing other investigations requiring attention at the time, it was impossible to obtain all the information desired on the pest and the natural enemies that control it, but it is hoped that the data which have been accumulated may be of service to entomologists and interested woodland owners.

One feature of the control of *Heterocampa guttivitta* should be emphasized and that is that only a minimum amount of hand work or spraying was practised in the woodlands, which gave practically all the credit for the disappearance of the outbreak to natural enemies. The list which follows gives the natural enemies found in connection with, preying upon, or bred from *H. guttivitta* Walk.:

Eggs	*Telenomus coelodasidis* Ashm.
	Trichogramma minutum Riley
Larvae	*Elachertes cacoeciae* How.
	Meteorus hyphantriae Riley
	Apanteles schizurae Ashm.
	Hyposoter clisiocampae Weed
	Campoplex sp.
	Compsilura concinnata Meig.
	Winthemia quadripustulata Fab.
	Admontia hylotomae Coq. (?)
	Actia sp.
	Calosoma frigidum Kirby
	Calosoma sycophanta L.
	Podisus modestus Dall.
	Mermis n. sp.
	Disease and starvation
Pupae	*Ephialtes* (*Ephialtes*) *pedalis* Cress.
	Amblyteles sublatus Cress.
	Enicospilus purgatus Say
	Calosoma frigidum Kirby
	Calosoma sycophanta L.
	Carabus sylvosus Say
	Cychrus elevatus Fab.
	Dysidius (*Pterostichus*) *mutus* Say
	Sorex personatus Geof.
	Blarina brevicauda Say

[3] The writer is indebted to the following coworkers at the Gipsy Moth Laboratory: S. S. Crossman and C. F. W. Muesebeck for determination of hymenopterous material; to Mr. Muesebeck for records of collections and rearings; to R. T. Webber and J. V. Schaffner, jr., for rearing records of hymenopterous and dipterous parasites, and to Mr. Webber for the determination of the latter; to C. E. Hood and F. W. Graham for collections and observations in the field; and to J. N. Summers and Miss Theresa Sheerin for examination of diseased material.

PARASITES OF THE EGG

Attempts were made to collect eggs of *Heterocampa guttivitta*, which in some seasons were difficult to obtain. A small number, however, was obtained in Tamworth, N. H., on June 15, 1920, which showed no parasitism, although it is possible that the collection was made too early for parasites to have visited the eggs. Collections in this section in 1921 showed the following results:

June 7, 1921, at North Conway, N. H. (Cramer Mountain), 8 eggs collected, which yielded 4 larvae of *H. guttivitta*, Four of these, or 50 per cent, contained parasites of *Telenomus coelodasidis* Ashm.

June 8, 1921, at North Conway, N. H. (White Horse Cliff), 11 eggs collected; all, or 100 per cent, parasitized by *T. coelodasidis*.

June 7, 1921, at Lower Bartlett, N. H., 52 eggs and 6 newly hatched larvae collected. The eggs contained 3 larvae of *H. guttivitta*, 46, or 88 per cent, parasitized by *T. coelodasidis*, 3 eggs were found to be infertile.

July 13, 1921, another collection of 55 eggs was made at the same place, and examinations showed the following:

One egg contained dead embryo of *H. guttivitta;* 4 eggs had hatched; 1 egg was infertile; 47 eggs, or 85 per cent, had yielded *T. coelodasidis* adults or contained same within chorion; 2 eggs yielded *Trichogramma minutum* Riley after July 15, 1921.

On June 24, 1922, another visit was made to the region of New Hampshire previously infested, and a diligent search was made for eggs and small larvae of *Heterocampa guttivitta*. Only four eggs were found, three of which were parasitized by *Telenomus coelodasidis* and one of which was infertile. In two locations where the three parasitized eggs were found no others were taken, indicating an approximate parasitism of 100 per cent in those localities. No hatched eggs or larvae were found. This visit showed a much further decrease in numbers of the host in 1922 than in 1921, a fact which was due principally to egg parasitism.

On June 21, 1923, the search for eggs was again repeated in New Hampshire but only three were found, one of which was parasitized by *T. coelodasidis*.

These records indicate that the maximum egg parasitism was reached in New Hampshire in 1921 and 1922. The former year, it will be recalled, was that following the extremely heavy defoliation by the host. The parasitism by *Telenomus coelodasidis* indicates that this species was the most powerful single agency of control in New Hampshire the year following the heavy defoliation. Furthermore, the records explain in a measure the reason for the difficulty that was met in July, 1921, in obtaining enough larvae of the host for a representative collection. Large larvae could not be found after a careful search in late July and August of 1922, 1923, 1924, and 1925.

PARASITES AND PREDATORS OF THE LARVAE

Elachertus cacoeciae How., *Meteorus hyphantriae* Riley, *and Apanteles schizurae* Ashm.—These species were bred from a collection of small host larvae made in the New Hampshire section in June, 1921. It is impossible to estimate the abundance of these parasites, owing to the difficulty experienced in rearing the host material, but it is probable that they were of considerable importance during the last period of the outbreak.

Hyposoter clisiocampae Weed.—This parasite has been bred from *Heterocampa guttivitta* larval material, and the cocoons have also been found occasionally in the leaf mold during examinations for pupae in the Massachusetts section. It was not found to be especially abundant.

Campoplex sp.—One specimen in the cocoon stage from which no adult issued was obtained from a dead host larva collected in the New Hampshire section July 13, 1921.

Compsilura concinata Meig.—This parasite proved to be well distributed, having been reared at various times from several collections of large larvae, both from the Massachusetts and New Hampshire sections. For the most part the records indicated that this parasite was not especially effective on this host.

Winthemia quadripustulata Fab.—One specimen of this species was reared from a collection of 41 large larvae made in Worthington, Mass., July 9, 1919. This species did not emerge from any other collections made in the Massachusetts and New Hampshire sections.

Admontia hylotomae Coq. (?).—From one large larva of *Heterocampa guttivitta* collected in the New Hampshire section July 13, 1921, there issued 13 small tachinid larvae, some of which formed puparia, but no adults were obtained. The determination of this parasite was made by C. T. Greene, of the United States National Museum, but since only the immature stages were available there is some doubt as to the correctness of the determination. This parasite may have played a considerable part in the control of the pest, for when the collection was made only a few of the host larvae could be found.

Actia sp.—From a collection consisting of 162 one-third to two-thirds mature host larvae made at Tamworth, N. H., July 20, 1920, there issued with *Compsilura concinnata* one specimen of *Actia* sp. This species was not obtained from other collections.

Calosoma sycophanta L. *and Calosoma frigidum* Kirby.—For data collected on these species see "Parasites and predators of the pupae."

Podisus modestus Dall.—This species was noted in some of the *Heterocampa guttivitta* colonies. At times a nymph was seen on tree trunks with a half-dead larva impaled on its beak. These were found in the New Hampshire section, but were never abundant.

Mermis n. sp.—A collection of 700 nearly full-grown larvae was made on Cramer Mountain, North Conway, N. H., July 29, 1919. From each of two of the larvae, which later died, there emerged a nematode which was forwarded to N. A. Cobb, of the Bureau of Plant Industry, United States Department of Agriculture, for determination. He found it to be an undescribed species. No nemas were obtained from any of the other collections made.

Disease and starvation.—Conditions responsible for epidemics of disease among insects frequently have a very close relation to starvation. This relation was apparent in the locations where the maximum number of dead larvae were found in the center of large areas which for all practical purposes were completely defoliated, making it difficult to separate the factors actually causing death of the larvae.

On August 5, 1919, a collection of larvae dead from disease was made in Hawley, Mass., which upon examination was found to contain bacteria, pigment, and polyhedra. Diseased material taken

at Tamworth, N. H., on July 30 of the same year was full of various forms of bacteria, large and small bacilli, cocci, and diplococci. The nature of the epidemic and its effectiveness in the field, together with the fact that some of the samples showed polyhedral bodies, indicate that the disease in its effects on the host is very similar to the "wilt" of the gipsy moth (*3*). However, since all the samples did not show polyhedra, and one sample from New Hampshire suggested the work of fungi and bacteria as stated above, the proportion of larvae dying from the various causes was not obtained.

These diseases among the larvae were present in the Massachusetts region during 1919, and probably during the previous years, but their effectiveness reached a climax the last of July, 1919, in the New Hampshire territory. The large larvae were found dead in masses at the bases of forest trees, the large trees having from 200 to 500 hanging to the lower trunks and around their bases. Fully 90 per cent of the larvae in the center of many badly defoliated areas died of disease and starvation, the mortality lessening in degree as one advanced toward the extreme edge of the infestation. Death resulting from these causes made them powerful controlling factors in the checking of *Heterocampa guttivitta* Walk. in New Hampshire during that season, probably equaling the work of all other natural agencies combined. Somewhat similar conditions prevailed in that region during 1920 as far as the diseases were concerned, but the parasites and predators were more effective, with the result that heavy defoliation did not occur the following year.

PARASITES AND PREDATORS OF THE PUPAE

Ephialtes (*Ephialtes*) *pedalis* Cress.—September 10, 1918, 55 pupae of *Heterocampa guttivitta* were collected in North Conway and Tamworth, N. H. From some of these there issued specimens of *Ephialtes* (*Ephialtes*) *pedalis* Cress. the following spring. This species was obtained in only one collection of pupae, but it is presumed that it was probably common but not especially abundant.

Amblyteles sublatus Cress., *and Enicospilus purgatus* Say.—*Amblyteles sublatus* was reared from several collections of large larvae from both the Massachusetts and New Hampshire sections. These larvae later pupated, and the parasite emerged from the pupae the following spring. Usually this parasite was recovered in rather small numbers, but from one collection of 28 pupae from Tamworth, N. H., June 9, 1920, there issued 13 specimens of this species and *Enicospilus purgatus* Say. This is the only record during the outbreak where the latter parasite was reared, but it was evident in this case that the two species working at the same time exacted heavy toll from the host.

Calosoma frigidum Kirby.—The first observations on natural enemies were made during the late summer, both in Massachusetts and New Hampshire. Leaf mold where the host insect pupates was carefully examined and the findings classified in somewhat the same manner as given in Tables I and II. The classification was not wholly satisfactory, but the same standard of comparison was used through the series of years (in that about the same number of square yards of leaf mold were examined) and it serves to illustrate especially the increase in numbers and effectiveness of this predator.

TABLE I.—*Number of pupae dead from parasitism or unknown causes, and those destroyed by Calosoma frigidum in Massachusetts, during 1918 and 1919*

Towns in which collections were made	Pupae collected in—		Pupae living in—		Dead from parasites or unknown causes		Destroyed by C. frigidum			
	1918	1919	1918	1919	1918	1919	1918		1919	
							No.	*P. ct.*	*No*	*P. ct.*
Cummington	16	7	6	2	6	1	4	25.0	4	57.0
Worthington (two locations)	28	57	9	[a] 17	7	3	12	43.0	37	65.0
Hawley	74	14	55	1	2	5	17	23.0	8	57.0
Heath	26	11	19	2	1	5	6	23.0	4	36.4
Colraine	18	----	10	----	2	----	6	33.3	----	----
Ashfield	27	----	20	----	5	----	2	7.4	----	----
Plainfield	49	20	48	10	1	2	0	.0	8	40.0
Middlefield	53	----	17	----	10	----	26	49.0	----	----
Becket	20	----	6	----	5	----	9	45.0	----	----
Charlemont	----	17	----	0	----	2	----	----	15	88.2
Total	311	126	190	32	39	18	82	26.4	76	60.3

[a] Observations made Aug. 5 before *C. frigidum* finished feeding. Forty beetle larvae and one adult were found in 8 square yards of leaf mold.

TABLE II.—*Number of pupae dead from parasitism or unknown causes, and those destroyed by Calosoma frigidum in New Hampshire, 1918 to 1920*

Towns in which collections were made	Pupae collected in—			Pupae living in—			Dead from parasitism or unknown causes			Destroyed by C. frigidum					
	1918	1919	1920	1918	1919	1920	1918	1919	1920	1918		1919		1920	
										No.	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*
Sandwich	10	60	----	8	40	----	[a] 2	[b] 18	----	----	----	2	3.3	----	----
Tamworth (two locations)	60	68	16	48	26	2	0	[b] 20	1	12	20	22	32.3	13	81.3
North Conway (two locations)	3	207	----	2	153	----	[a] 1	[b] 27	----	----	----	27	13.0	----	----
Albany (two locations)	----	61	23	----	49	0	----	[b] 9	4	----	----	3	4.9	19	82.6
Bartlett (two locations)	----	48	10	----	30	3	----	[b] 13	1	----	----	5	10.4	6	60.0
Total	73	444	49	58	298	5	3	[b] 87	6	12	16.4	59	13.3	38	77.6

[a] Pupae had the appearance of having been destroyed by small carabids.
[b] Dissections were made of partial lots of these pupae, this proportion of the whole containing larvae of tachinids.

It will be noted from Table I that the average percentage of effectiveness of *Calosoma frigidum* in Massachusetts in 1919 was more than twice that of 1918. It is thought that this increased effectiveness was not wholly due to the increase in numbers of beetles present during the latter year, but rather to the decrease in intensity of infestation in 1919 as a result of various causes. The tables do not show the work of the adults on the larvae of *Heterocampa guttivitta*, which was an important factor (judging from the large numbers of beetles sometimes seen), but only of the larvae of the predator on the pupae. It is probably fair to assume that the percentage of *H. guttivitta* larvae destroyed by adults of *C. frigidum* during each larval season equaled the percentage of pupae destroyed by these predatory larvae. Such an assumption is based upon numerous field records showing the quantity of food consumed by *Calosoma sycophanta* as an enemy of *Porthetria dispar* L. in New England.

The rise in the effectiveness of *Calosoma frigidum* in New Hampshire, as shown by Table II, was somewhat different from that recorded in Massachusetts. In 1918, when the infestation was rather light but rapidly increasing, the work of *C. frigidum* was found in one town out of three to cause an average destruction of 16.4 per cent of the pupae. The following year, when the area of infestation increased and many thousands of acres were defoliated, with many more pupae in the leaf mold, the average percentage of those destroyed fell to 13.3 per cent. The third year (1920), when the damage to the forests was equally as great or greater than in 1919, the average percentage of pupae destroyed by *C. frigidum* reached 77.6 or six times that of the previous year. From these data it is evident that *C. frigidum* was one of the most powerful agents in the control of the host, at least in the last two years of the outbreak. It is also apparent that countless thousands of the beetles and their larvae died of starvation during the closing periods of the outbreak.

Calosoma sycophanta L.—This species was found in a few colonies of *Heterocampa guttivitta*, both in the Massachusetts and New Hampshire sections, but not in sufficient numbers to figure effectively in control.

Carabus sylvosus Say.—One specimen was found in leaf mold while pupae of *Heterocampa guttivitta* were being collected at Tamworth, N. H., September 21, 1920. Its presence with the pupae was not positive proof that it had been preying upon them.

Cychrus elevatus Fab. and *Dysidius* (*Pterostichus*) *mutus* Say.—One specimen of the former was found in leaf mold with *Heterocampa guttivitta* pupae at Tamworth, N. H., September 11, 1918. At the same time two pupa cases were discovered which looked as if they had been fed upon by small carabids. *Dysidius* (*Pterostichus*) *mutus* Say was also found under like conditions another year.

Sorex personatus Geof. and *Blarina brevicauda* Say.—During several fall examinations of leaf mold in the New Hampshire section in 1919 and 1920, runways of small animals similar to those made and used by the shrews *Sorex personatus* Geof. and *Blarina brevicauda* Say were frequently noted, and particles of *Heterocampa guttivitta* pupa cases were strewn about the leaf mold, indicating inroads of considerable consequence. None of these animals were captured, and their exact species could not be determined. It is possible that the destruction of pupae noted may have been caused by *Evotomys* sp.

CONCLUSION

Attempts have been made to chronicle the important phases of the recurring outbreaks of *Heterocampa guttivitta* and to list the enemies that were responsible for its reduction to a minimum in Massachusetts in 1919 and in New Hampshire in 1920. Only small numbers of the host were left to hibernate at the end of these years to produce a crop of moths the succeeding year. The enemies most prominent in reducing the numbers and ravages of the host were: Epidemics of disease, together with starvation among the larvae; *Calosoma frigidum* Kirby; the egg parasite *Telenomus coelodasidis* Ashm. and several of the hymenopterous and dipterous parasites working simultaneously on the larvae and pupae.

Heterocampa guttivitta, with its phenomenal outbreaks followed by periods of comparative rarity, presents a most interesting problem in insect parasitism. It has been followed through two serious outbreaks in New Hampshire at 11-year intervals, but definite knowledge of the parasites which were most influential in controlling it during the intervening years is limited, owing to the fact that its history could not be followed continuously. If similar conditions prevail in the future as those which governed the outbreak in the period 1917 to 1920 and the period which preceded it, the infestation may be expected to recur in 1928 to 1930.

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